Trace Element Analysis of Food and Diet

RSC FOOD ANALYSIS MONOGRAPHS

Series Editor: P.S. Belton, *School of Chemical Sciences*, *University of East Anglia*, *Norwich*, *UK*

The aim of this series is to provide guidance and advice to the practising food analyst. It is intended to be a series of day-to-day guides for the laboratory worker, rather than library books for occasional reference. The series will form a comprehensive set of monographs providing the current state of the art on food analysis.

OTHER TITLES IN THIS SERIES:

Chromatography and Capillary Electrophoresis in Food Analysis
By H. Sorensen, S. Sorensen and C. Bjergegaard, *Royal Veterinary and Agricultural University Frederiksberg*, *Denmark* and S. Michaelsen, *Novo Nordisk A/S*, *Denmark*

Dietary Fibre Analysis
By D.A.T. Southgate, *Formerly of the AFRC Institute of Food Research*, *Norwich*, *UK*

Mass Spectrometry of Natural Substances in Food
By F. Mellon, *Institute of Food Research*, *Norwich*, *UK,* R. Self, *University of East Anglia*, *Norwich*, *UK* and J.R. Startin, *Central Science Laboratory*, *York*, *UK*

Quality in the Food Analysis Laboratory
By R. Wood, *MAFF*, *Norwich*, *UK,* H. Wallin, *VTT Biotechnology and Food Research*, *Finland,* and A. Nilsson, *National Food Administration*, *Sweden*

The Maillard Reaction
By S.E. Fayle, *Crop and Food Research*, *New Zealand* and J.A. Gerrard, *University of Canterbury*, *New Zealand*

Extraction of Organic Analytes from Foods: A Manual of Methods
By R. Self, *University of East Anglia*, *Norwich*, *UK*

How to obtain future titles on publication

A standing order plan is available for this series. A standing order will bring delivery of each new volume upon publication. For further information please contact:

Sales and Customer Care
Royal Society of Chemistry, Thomas Graham House
Science Park, Milton Road, Cambridge, CB4 0WF, UK
Telephone: +44(0)-1223-420066, Fax: +44(0)-1223-426017,
Email: sales@rsc.org

Trace Element Analysis of Food and Diet

Namık K. Aras
Middle East Technical University, Retired
Turkish Academy of Sciences, Member
Ankara, Turkey

O. Yavuz Ataman
Middle East Technical University
Ankara, Turkey

RSC Publishing

Cover image based on an image courtesy of USDA-ARS

ISBN-10: 0-85404-576-7
ISBN-13: 978-085404-576-1

A catalogue record for this book is available from the British Library

Published by The Royal Society of Chemistry,
Thomas Graham House, Science Park, Milton Road,
Cambridge CB4 0WF, UK

Registered Charity Number 207890

For further information see our web site at www.rsc.org

Typeset by Macmillan India Ltd, Bangalore, India
Printed by Henry Ling Ltd, Dorchester, Dorset, UK

Preface

The researchers who choose to work in the field of trace element determinations are not necessarily experienced analytical chemists. However, once involved in this sort of research, they either should acquire the necessary instrumentation in their laboratory or should be able to communicate with their collaborating colleagues who will probably be analytical chemists. In any case, this type of reader will need to know more about analytical chemistry, its language, literature and basics. Some chapters of this book will address this class of reader who need a rather quick review of the field through easy reading.

The book should also be useful to readers who perform actual experiments for sampling, analysis and evaluation. Therefore, especially the last chapter will provide the reader with procedures, brief suggestions for methodology and current references. All chapters include illustrations. These are mostly adapted from original articles or literature developed by manufacturing companies. Therefore, our choice of this particular approach is intended to establish some useful linkages between theory and actual practices in the manufacturing world.

The language, style and appearance of the book have been designed carefully by the authors who both have over thirty years of teaching and research experience in the field of analytical chemistry that hopefully has contributed to the pedagogical aspect of the book. This book is expected to provide an easily comprehensible basic orientation for those new in the field while at the same time offering ample opportunities for experienced researches to acquire new perspectives.

Some parts of Chapter 9, Nuclear Activation Analysis, have been based on the lecture notes of N.K. Aras and D.L. Anderson, which were prepared while they were giving a short course at the University of Maryland. Namık Aras would like to thank to late Professor Glen E. Gordon who taught him the importance of trace elements during his years at MIT and University of Maryland and to Robert Parr from IAEA for many years of fruitful discussions on trace elements in diet. Thanks are also due to R. Lindstrom from NIST and M. Yukawa from National Institute of Radiological Sciences, Japan for providing gamma ray and PIXE spectra of diet samples, and Özge Hacıfazlıoğlu for helping us in organizing the index of this book. Special thanks go to Peter Belton who encouraged us to write this book; and Annie Jacob, Janet Freshwater and Katrina Turner from the RSC for their organizational help. Finally we thank our wives Çiğdem Aras and Gülay Ataman for their moral support and patience throughout this endeavor.

Namık K. Aras and O. Yavuz Ataman
January, 2006

Contents

Abbreviations

AAS	Atomic absorption spectrometry
AES	Atomic emission spectrometry
AFS	Atomic fluorescence spectrometry
AgDDC	Silver diethyldithiocarbamate
AOAC	Association of Official Analytical Chemists
APDC	Ammonium pyrrolidine dithiocarbamate
AsB	Arsenobetaine
CCD	Charge coupled device
CE	Capillary electrophoresis
CEC	Capillar electrochromatography
CID	Charge injection device
CM	Chemical modifier
CRM	Certified reference material
CTD	Charge transfer device
CV-ICP-MS	Cold vapour inductively coupled plasma mass spectrometry
DAN	Diaminonaphtalene
DBT	Di-butyl tin
DCP	Direct current plasma
DDTC	Diethydithiocarbamate
DL	Detection limit
DMA	Dimethylarsinate
ECD	Electron capture detector
EDXRF	Energy dispersive X-ray fluorescence
EG	Electrochemical generation
EIE	Easily ionizable elements
ES	Electrospray
ETAAS	Electrothermal atomic absorption spectrometry
ETV	Electrothermal vaporizer
FAAS	Flame atomic absorption spectrometry
FAES	Flame atomic emission spectrometry
FAFS	Flame atomic fluorescence spectrometry
FI	Flow injection
FID	Flame ionization detector
FT	Fourier transform
FWHM	Full width at half maximum

GC	Gas chromatography
GC-AES	Gas chromatography-atomic emission spectrometry
GD	Glow discharge
GFAAS	Graphite furnace atomic absorption spectrometry
GLC	Gas liquid chromatography
HEPA	High efficiency particulate air
HG	Hydride generation
HGAAS	Hydride generation atomic absorption spectrometry
HG-AFS	Hydride generation atomic fluorescence spectrometry
HG-ICP-MS	Hydride generation Inductively coupled plasma mass spectrometry
HG-ICP-OES	Hydride generation Inductively coupled plasma optical emission spectrometry
HPLC	High pressure liquid chromatography
IBMK	Isobutylmethylketone
IC	Ion chromatography
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
ICR-MS	Ion cyclotron resonance mass spectrometry
ID	Isotope dilution
IEC	Ion exchange chromatography
IFNAA	Instrumental fast neutron activation analysis
INAA	Instrumental neutron activation analysis
IR	Infrared
ISE	Ion selective electrode
IUPAC	International Union of Pure and Applied Chemistry
LA	Laser ablation
LC	Liquid chromatography
LEAF	Laser excited atomic fluorescence
LOD	Limit of detection
LOQ	Limit of quantitation
MAA	Molecular activation analysis
MALDI	Matrix assisted laser desorption ionization
MIBK	Methylisobutylketone
MIP	Microwave induced plasma
MIP-AES	Microwave induced plasma - atomic emission spectrometry
MM	Matrix modifier
MMA	Monomethylarsonate
MS	Mass spectrometry
MW	Microwave
MWD	Microwave digestion
NAA	Neutron activation analysis
NIST	National Institute of Standards and Technology
OES	Optical emission spectrometry
PDA	Photodiode array

PFA	Perfluoroalkoxyfluorocarbon
PGAA	Prompt gamma activation analysis
PIXE	Particle induced X-ray emission
PTFE	Polytetrafluoroethylene
Q	Quadrupole
QA	Quality assurance
QC	Quality control
QMA	Quadrupole mass analyzer
REE	Rare earth elements
RF	Radio frequency
RSD	Relative standard deviation
S/N	Signal to noise ratio
SDS-PAGE	Sodium dodecylsulfate polyacrylamide gel electrophoresis
SEC	Size exclusion chromatography
SFC	Supercritical fluid chromatography
SP	Spectrophotometry
SPME	Solid phase micro extraction
SR	Synchroton radiation
SRM	Standard reference material
SRXRF	Synchroton radiation X-ray fluorescence
SSMS	Spark source mass spectrometry
SXRF	Synchroton X-ray fluorescence
TBT	Tributyl tin
TCA	Trichloroacetic acid
TCD	Thermal conductivity detector
THF	Tetrahydrofuran
THGA	Transversely heated graphite atomizer
TIMS	Thermal ionization mass spectrometry
TMAH	Trimetylammonium hydroxide
TOF	Time of flight
TOF-MS	Time of flight mass spectrometry
TXRF	Total reflection XRF
USN	Ultrasonic nebulisation
UV	Ultraviolet
VIS	Visible
WDXRF	Wavelength dispersive X-ray fluorescence
XRF	X-ray fluorescence

CHAPTER 1

Introduction

1.1 Importance of Trace Elements in Food

1.1.1 Essential Trace Elements

Food and beverages ingested by humans represent a potentially proficient pathway of exposure to toxic and nutritionally important minor and trace elements. Many mineral elements occur in living tissues, food and diets in such small amounts that they are frequently described as "traces" and the phrase "trace elements" arose to describe them. At the present time, less than one-third of the 90 naturally occurring elements are known to be essential for life.

The bulk of human body is composed of six major elements; oxygen, carbon, hydrogen, nitrogen, calcium and phosphorus and six minor elements; sulfur, potassium, sodium, chlorine, magnesium and silicon. The total percentage of minor and major elements in total body weight is 98.8 (Table 1.1).[1] If six noble gases are excluded as unlikely to have a physiological function, 71 elements of the periodic system remain, and because of their low concentration in living matter, are termed the "trace elements".

The concentration of major and minor elements in living tissues can be expressed in grams per kilogram. On the other hand, the concentration of trace elements in living tissues varies between 0.01 and 100 mg kg^{-1} (Table 1.2). It may not be appropriate to classify them as essential or toxic elements. It is logically wrong to establish a category of "toxic" elements, because any element may be potentially toxic and this property is but a function of concentrations to which humans are exposed. Essentiality of the trace elements is established when a further reduction below the range of tolerable levels, better known as "range of safe and adequate intakes", results in a consistent and reproducible impairment of a physiological function.[2,3]

These considerations suggest a logical classification of the 71 trace elements into those, with proven essentiality and the rest for which essentiality is "presently not known". This classification leaves room for the possibility that future research will include additional elements as essential. Each of the two categories can be subdivided according to their practical importance under given conditions; *e.g.*, local, regional or national imbalance in the environment, industrial emissions or dietary habits. Some essential elements may not be of any nutritional concern at all, as in

Table 1.1 *Concentrations of major and minor elements in reference man (percent in total body weight)*

Major elements	*Percent (%)*	*Minor elements*	*Percent (%)*
O	61	S	0.2
C	23	K	0.2
H	10	Na	1.4
N	2.6	Cl	1.2
Ca	1.4	Mg	0.03
P	1.1	Si	0.03

Table 1.2 *Range of concentrations of trace elements in human body*

Element	*mg kg^{-1}*
Fe, F, Zn	100.0
Rb, Sr, Cu, Pb, Br	10.0
Sn, Sc, Cd, Mn, Ba, Al	1.0
Cs, Co, Cr, Mo, Au, Ni	0.1

the case of magnesium in human nutrition, since it is in sufficiently high levels; others, such as selenium, may have the highest regional importance because of deficiency in one area and toxicity in another.

In recent years, there has been an increase in the realization of the importance of the role of trace elements in biological systems. The study of life processes shows that many vital functions are dependent on the presence of a specific trace element. Because of that, trace elements are one of the important nutrient factors for the growth and maintenance of human and animal life.

Food only, excluding intakes from water and air, normally supplies a major proportion of the total daily trace element intake by humans. Since the late 1950s, concerns over the introduction of trace elements and many other components into the environment as a result of human activities have greatly increased. Besides soil and water, food is also contaminated with trace metals by the introduction of mechanized farming, ever increasing use of chemicals, sprays, preservatives, food processing and canning. In order to get the minimum adverse impact, it is important to measure and continuously monitor their levels in various food items, total diet, water and inhaled air.

The concentrations of trace elements in food give important information about dietary habits of special group, health situation of individuals and origins of elements. Therefore, it is important to determine the daily dietary intake of trace elements, their concentrations and sources.

Recent developments of trace element research in the area of nutrition have led to a need to accurately and precisely determine the content of these micronutrients in food. In the past several decades, the analytical chemistry community has made great advances in improvement in sensitivity, selectivity and accuracy of analytical methodology.

In this book, we will present experimental techniques for the collection, preparation and determination of trace elements in food. All the modern techniques will be discussed in some detail so that it will be useful for both researcher and technical staff who are working in this area.

1.1.2 Classification of Trace Elements

The simplest definition of trace essential element is that it is required in small amount for the maintenance of life; its absence results in death or a severe malfunction of the organism.

All major and minor elements are important; besides that, some of the trace elements e.g; Cr, Fe, Co, Cu, Zn, Se, Mo and I are essential trace elements; and some of them; Mn, Si, Ni, B, V, and Sn are probably essential trace elements; and further some of them F, As, Cd, Pb, Al and Hg are considered potentially toxic, some possibly essential elements for animal and human life. Actually all essential elements may also be toxic in animals and humans if ingested at sufficiently high levels and for a long enough period (Fig.1.1)[4]. The above elements will be discussed in detail in Chapter 13.

Essential trace elements are required by man in amounts ranging from 50 μg day^{-1} to 20 mg day^{-1}. The organism can neither grow nor complete its life cycle without the element in question. The element should have a direct influence on the organism and be involved in its metabolism. The effect of the essential element cannot be wholly replaced by any other element.

The bioavailibilities of the essential elements depend on their chemical form, the compositions of diet and health situation of the individuals. Thus, establishment of the optimum daily requirements and determination of actual daily intake of essential elements are important problems of trace element in nutrition.[5]

The essential trace elements provide a classical example of required nutrients as described by Bertnard as early as 1911. An organism may go through several stages as the concentration of essential nutrient progresses from deficiency to excess. In absolute deficiency, death may result, with limited intake; the organism survives but may show marginal insufficiency. With increasing nutrient, a plateau representing the optimal function is reached. As the nutrient is given in excess, first marginal toxicity then mortal toxicity are attained while this curve may vary quantitatively for each essential nutrient, the basic pattern holds for virtually all the essential trace elements. This is illustrated in Figure 1.2 for selenium. There is barely a fourfold range between intake per day for survival and that for the appearance of toxic effects.[5]

1.1.3 Discovery of Essential Trace Elements

The study of the discovery of essential trace elements has been outlined by Schrauzer.[6] The treatment of anaemia with iron and the association of iodine deficiency with goiter marked these as the only two essential trace elements recognized for animals before the twentieth century. In the twentieth century, there were two major periods of activity in biological trace element research. In the early classical period, 1925–1956, the essentiality of copper, zinc, cobalt, manganese and molybdenum in animals was discovered. A more active modern period, 1957–1980, dominated by the late Klav Schwarz, was

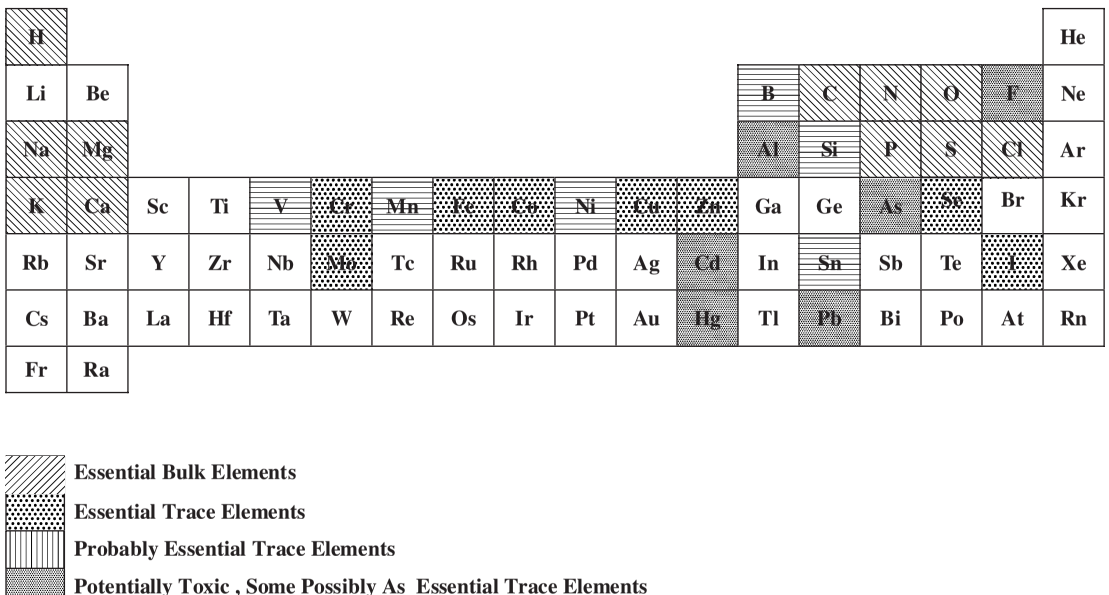


Figure 1.1 *Present status of the essentiality of the elements in the periodic table. The 12 bulk elements and 18 trace elements are identified as essential*

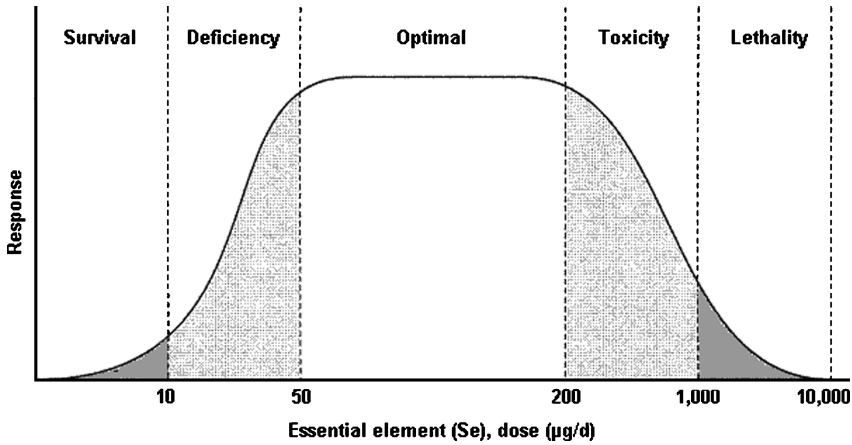


Figure 1.2 *Dose–response range of an essential element. Estimates of specific requirements in terms of micrograms per day for selenium*

marked by the experimental induction of trace element deficiencies. These efforts have resulted in evidence supporting the essentiality of selenium, chromium, tin, vanadium, fluorine, silicon, nickel, lead, cadmium, arsenic and most recently lithium.

1.1.4 Functions of Trace Elements

Most of the trace elements serve a variety of functions, depending upon their chemical form or combination and their location in the body tissues and fluids.

Minor and trace elements serve in two general roles. The first one is their function as structural material. Iron is part of the structure of the oxygen-carrying protein, haemoglobin, in the red blood cells; calcium, phosphorus and other elements constitute a significant part of the mass of teeth and bones; and sodium, potassium, phosphate, sulfate, chloride and many other elements are important constituents of the fluids, both inside and outside all the body cells.

The second general role of trace elements is their function in regulating numerous biological activities. Calcium in minute concentrations is necessary for normal blood clotting; magnesium stimulates the activity of many enzymes and a number of trace elements control the contraction of muscle and the transmission of impulses by nerve cells. Table 1.3 lists the macrominerals and trace elements known to be essential in human nutrition and their functions.[5,6]

1.2 Trace Element Studies

The study of trace element contents in food, environmental and biological samples has attracted worldwide interest, and a lot of papers are published in this field. Since early 1970s, there has been an increasing interest in the levels of several elements in composite diet and individual food items such as honey, meat, milk, wheat, water,

Table 1.3 *Functions of essential macrominerals and trace elements*

Element	*Chief functions in the body*
Calcium	Principal constituent of bones and teeth: involved in muscle contraction and relaxation, nerve function, blood clotting, blood pressure.
Phosphorous	Part of every cell: involved in pH buffering
Magnesium	Involved in bone mineralization, protein synthesis, enzyme action, normal muscular contraction, nerve transmission.
Sodium	Helps maintain ionic strength of body fluids
Chloride	Part of stomach acid, necessary for proper digestion
Potassium	Facilitates many reactions, including protein synthesis, nerve transmission and contraction of muscles.
Sulfur	Component of certain aminoacids, part of biotin, thiamin and insulin.
Iodine	Part of thyroxin, which regulates metabolism
Iron	Haemoglobin formation, part of myoglobin, energy utilization.
Zinc	Part of many enzymes, present in insulin, involved in making genetic material and proteins, immunity, vitamin A transport, taste, wound healing, making sperm, normal fetal development
Copper	Absorption of iron, part of several enzymes
Fluoride	Formation of bones and teeth, helps make teeth resistant to decay and bones resistant to mineral loss
Selenium	Helps protect body compounds from oxidation
Chromium	Associated with insulin and required for the release of energy from glucose
Molybdenum	Facilities enzyme functions and many cell processes
Manganese	Facilities enzyme functions and many cell processes
Cobalt	Part of vitamin B_{12}, which involves in nerve function and blood formation
Vanadium	Control of sodium pump: inhibition of ATPase, *p*-transferases
Nickel	Constituent of urease, reduced haemopoiesis
Cadmium	Stimulates elongation Betois in ribosomes
Tin	Interactions with riboflavin
Lead	Many enzyme effects
Lithium	Control of sodium pump
Silicon	Structural role in connective tissue and osteogenic cells
Arsenic	Increased arginine urea + ornithine, Meto, metabolism of methyl compounds
Boron	Control of membrane function, nucleic acid biosynthesis and lignin biosynthesis

fish and vegetables.[7,8] Also a great deal of research has been undertaken on the concentration of essential trace elements in biological materials such as fluids and tissues. Attempts have been made in recent years to understand the role of trace elements in biological system, particularly in human metabolism.

The results obtained by the analyses of the trace elements in foods may not show the exact elemental values taken by human daily that may be lost due to contamination during washing, cooking and eating procedures.

References

1. K. Heydon, *Proceedings of the First International Conference on Elements in Health and Disease*, Vol 6, New Delhi, India, 1983, 42.
2. W. Mertz in *Trace Elements in Human and Animal Nutrition*, W. Mertz (ed), Academic Press, 1987, **1**, 1.

3. EPA Guidelines for Exposure Assessment, *Fed. Regst*., 1986, **51**, 34046.
4. "Trace Elements in Human Nutrition and Health", WHO, Geneva, 1996.
5. E. Frieden, *J. Chem. Educ*., 1985, **62**, 11, 917.
6. G.N. Schrauzer, *Biochem. Of the Essential Ultratrace Elements*, E. Frieden (ed), Plenum press, New York, NY, 1984, 17.
7. M.A. Boyle and G. Zayla, *Personal Nutrition*, 2nd edn, West Publishing Company, St. Paul, New York, Los Angeles, San Francisco, 1992.
8. N.K. Aras and I. Olmez, *Supp. Nutr.*, 1995, **11**, 506.

CHAPTER 2

Statistical Evaluation of Data

2.1 Introduction

Statistics is concerned with the organization, analysis and interpretation of numerical data. Since many results are obtained during trace element analysis of food and diet, statistical evaluation of data is most important. An analyst should know how to describe the results of the measurements, understand the statistics used in evaluation of the data, has to interpret the various types of data and make the basic statistical computations. The statistical evaluations are mostly used for

(1) measuring the central tendency,
(2) measuring the variability and
(3) measuring the relationship between different measurements.

The first two tasks provide a convenient means of analysing and describing a single set of data, and the last one can be used to indicate the agreement between data from different sources or different data sets.

2.2 Analytical Errors

2.2.1 Accuracy and Precision

In most chemical analyses, the true value is not known and error arises from the method, instruments, *etc*. Therefore, statistical analysis has to be used to determine the errors and to obtain the reasonable expression of results. In calculations, it is necessary to make a distinction between the exact and approximate values. Most of the results are approximate, since an interval and not exact points on some scale represent them. For example, if a food sample weighed for trace element analysis is 0.056 g, then it is expected that its value will be between 0.055 and 0.057 g. The deviation from the exact value is expressed in terms of *accuracy*, which can be defined as the correctness of a measurement or the nearness of a measurement to the true value. If, for example, a true value is μ and experimental value x_i, then the difference between the two values is the absolute error,

$$\text{absolute error} = x_i - \mu \tag{2.1}$$

The error is a measure of the accuracy of that determination. In practice, the error is often expressed in terms of percent relative error,

$$\text{percent relative error} = \frac{(x_i - \mu) \times 100}{\mu} \tag{2.2}$$

Thus, the accuracy of a measurement is often expressed in terms of percent relative error.

In most analysis, the actual value of measurement is not known with any degree of exactness. However, the agreement between the repeated measurements should still be satisfactory. This is expressed in terms of *precision*, which can be defined as the measure of the reproducibility of a measurement.

Accuracy and precision are different characteristics of a set of measurements, and they should be correctly interpreted. Accuracy expresses the correctness, and precision is the reproducibility of a measurement. A good precision does not mean a good accuracy, because it is possible to repeat the same error systematically for a measurement. However, for an acceptable measurement, both the precision and accuracy should be reasonably good.

2.2.2 Determinate and Indeterminate Errors

Absolute error is the difference between a measured value and the true value Equation (2.1). In an experiment the errors may be classified as determinate (systematic) and indeterminate (random).

Determinate errors have definite values with positive or negative directions; their sources can be found and the error can often be corrected. Therefore, they have a rather constant nature from one measurement to another. The most common determinate errors are due to improper calibration of instruments and use of instruments by an inexperienced or careless person. Also a colour-blind person cannot accurately differentiate between colours during a titration where visual indicators are used. If the method chosen is not suitable for the analysis, a serious error will be obtained which cannot be corrected easily. For example, if a gravimetric method is used for an analyte, which does not have a small solubility product, the results will be inaccurate.

Indeterminate errors are experimental errors, as a result of small differences in replicated results. This type of error is not systematic and cannot be corrected. The most important source of random errors may be the result of unknown inhomogeneity of the sample, impurities in the sample, instrumental fluctuations, imperfections in the experimental technique and fluctuation in experimental conditions, such as temperature, conductivity, electrical voltage.

In almost every experiment there may be some error, which have to be corrected either directly or statistically. The determinate error usually gives the degree of accuracy, whereas indeterminate error gives the degree of precision. The accuracy is dictated mostly by determinate errors where the precision is a function of indeterminate errors.

2.2.3 Significant Figures

Significant figures are the digits in a number including all the digits known with certainty plus the first one of the estimated digits. In calculations, the numbers are

usually rounded off to a lower number of significant figures as limited by the input value. The number in question can be an integer or fractional number. Thus, when a number is rounded off to have smaller number of significant figures, and if the disappearing digit is 0–4, the last digit of the result remains the same; when the disappearing digit is 6–9, the last digit is increased by 1. When the disappearing digit is 5, and if the last digit is an odd number, it is increased by 1; if it is an even number, it will be kept as it is. For example, when rounding the numbers down to three significant figures, 6.632, 6.638, 6.635 and 6.645 become 6.63, 6.64, 6.64 and 6.64, respectively.

The uncertainties in most of the analytical measurements depend on the instruments used. For example, an analytical balance, which has a precision of 0.1 g, can read a value such as 4.4 ± 0.1 g, where a balance with a precision of 0.1 mg will read 4.4615 ± 0.0001 g. Large numbers are expressed in powers of 10 to make the calculation simpler. However, the significant figures have to be considered in this form. For example, the weight 1245 mg can be written as 1.245×10^3 mg, but 3870.0 mg has to be written as 3.8700×10^3 mg.

In calculations, the significant figures have to be considered to obtain realistic results. In addition and subtraction type calculations, the number of significant figures is determined by the location of the decimal point and can be seen by visual inspection. Here, the input value with the smallest number of digits after the decimal point is limiting. However, it is best to retain all the digits until the arithmetic operation ends; the result will then be rounded. For example,

362.2
18.225
+5.3062
385.7312

Since the limiting number is 362.2, the result should be rounded to 385.7.

In multiplication or divisions, the number of significant figures in the resulting value will have the number of significant figures, which is limited by the input value with the lowest number of significant figures. If calculation contains both exact and approximate numbers, the number of significant figures in the result is determined by the number of significant figures in the approximated number. Therefore the molecular weight of N_2 is $2 \times 14.0067 = 28.0134$ but not 3×10^1.

In log terms, the result should have a number of significant figures, which equals to the number of digits before the exponential plus the number of digits appearing as the power of 10. For example, the pH of 3.4×10^{-9} M H^+ is 8.47. The first digit (8) comes from exponent (10^{-9}) and fraction, 0.47 from two significant figures of 3.4.

2.3 Mean, Median, Mode, Range and Mean Deviation

The prediction of the best value from experimental results can be done by calculating the central tendency of the set of results. There are four types of central tendencies in common use: mean, median, mode and range.

2.3.1 Mean

The mean, sometimes called arithmetic mean or average, is the sum of the separate results, x_i, divided by number of measurements, N:

$$\bar{x} = \frac{\Sigma x_i}{N} \tag{2.3}$$

The geometric mean is calculated by multiplying all the results, Πx_i, where Π indicates that one takes the product of the N values of x_i, and taking the power of $(1/N)$:

$$x_g = \sqrt[N]{\Pi x_i} \tag{2.4}$$

The geometric mean will be discussed in detail in Section 2.4.1.

2.3.2 Median

When the results of the measurements are arranged in ascending order, the median is the midpoint in the series. In this case, the number of results in series, N, can be even or odd. If N is odd, it is easy to find the midpoint. If N is even, the median is the average of two results at the midpoint. The median is used for the following cases: (1) when the exact midpoint of the distribution is required and, (2) when there are extreme results, median may have a better representation of the set as compared to mean.

2.3.3 Mode

In a series of measurements the mode is that single result which occurs most frequently. For example, in a set, which has result of 14, 15, 14, 12, 16, 11, 14, 13 and 15, the mode of the series is 14, since it has the highest frequency of occurrence.

2.3.4 Range

The range, W, is the interval or distance between the highest and the lowest values of a set of data. It is a good indication of scattering, and is very useful for rough comparisons of different sets of data. It is not a good measure of deviation or distribution of data when the data contain some extreme results.

2.3.5 Mean Deviation

The mean deviation, MD, is the average of the deviations of all the separate measurements in a series taken from their mean. If the mean for measurements x_i is $\bar{x}$ and total number of data, N, then MD for the data will be

$$\text{MD} = \frac{\Sigma(x_i - \bar{x})}{N} \tag{2.5}$$

Let us explain all these terms with an example.

The zinc content of a total diet sample was determined by instrumental neutron activation analysis in five different subsamples and the following N results were obtained in mg Zn/kg total diet: 27.1, 30.5, 28.6, 29.3 and 29.5.

The following were calculated:

(a) mean $= \Sigma x_i/N = (27.1 + 30.5 + 28.6 + 29.3 + 29.5)/5 = 29.0$.
(b) geometric mean $= \sqrt[N]{\Pi x_i} = 29.0$.
(c) median $= 29.3$.
(d) range $= 30.5 - 27.1 = 3.4$.
(e) mean deviation $= \Sigma(x_i - \bar{x})/N = 0.92$.

As seen, mean, geometric mean and median are all very close to each other. This indicates that as will be discussed below, the distribution was a Gaussian one.

2.4 Normal Distribution of Random Variables: Gaussian Distribution

The curve given in Figure 2.1 is called, Normal, bell or Gaussian curve. The vertical axis shows the relative frequency of occurrence of a measurement x_i or its error $x_i - \bar{x}$. In theory, if the number of measurements are infinite or very large, then the mean will be *population mean*, μ. But, in practice, the number of measurements will be limited, the mean will be *sample mean*, $\bar{x}$. The important part is the area under the curve, and the information that can be obtained about the population mean from the sample mean.

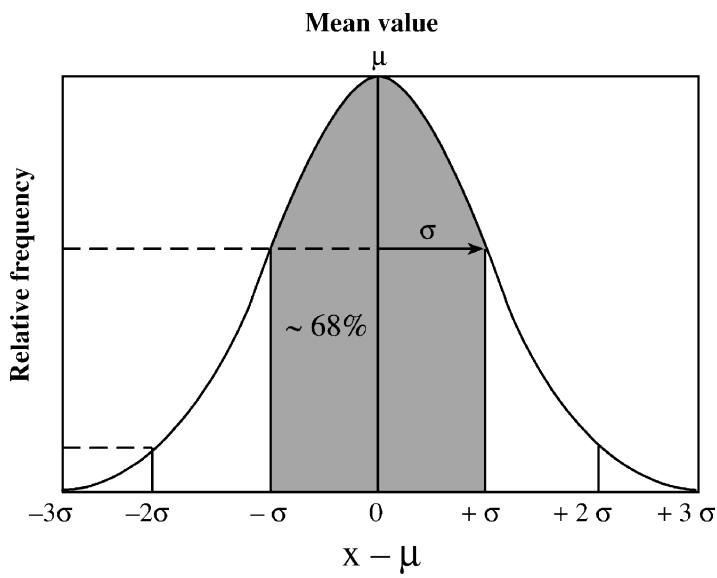


Figure 2.1 *Gaussian distribution*

This function, which is the fundamental distribution in statistics and theory of errors, leads to the following probability density equation:

$$y = \frac{1}{(\sigma\sqrt{2\pi})}\exp\left[-(x_i - \mu)^2/2\sigma^2\right] \tag{2.6}$$

where y is the frequency of a given x_i value, σ the standard deviation, μ the true value and $(x_i - \mu)$ the deviation from the true value or error. If the number of measurements is very large, in practice more than 20, we may assume the average $\bar{x}$ is equal to true value μ, provided that there is no systematic error. If the number of measurements is less than 20, s is used instead of σ.

The standard deviation of measurements illustrates how closely all measurements would cluster about the mean. The normal distribution curve gives information about the normal random error. Also the curve has a maximum value at $\bar{x}$, it is symmetrical with respect to $\bar{x}$ value, any change in the value of $\bar{x}$ changes the normal curve along the x-axis but the shape of the curve is not affected. Finally, a modification of σ will either widen or narrow the peak but $\bar{x}$ will be left unchanged. The equation can be modified by defining a new term, the z factor,

$$z = \frac{x_i - \mu}{\sigma} \tag{2.7}$$

The quantity z gives the deviation from the mean in units of standard deviation. The equation of distribution will then be

$$y = \frac{1}{\sigma\sqrt{2\pi}}\exp(-z^2/2) \tag{2.8}$$

The ideal curve of Equation (2.8), represented in Figure 2.1, is based upon an infinite number of observations with positive and negative deviations equally probable.

The measures of variability include certain constant fractions of total area of the normal curve. When the mean is taken as the centre, $\pm 1\sigma$ covers 68.26%, $\pm 2\sigma$ covers 95.46% and $\pm 3\sigma$ covers 99.74% of total area. The middle 50% corresponds to $\pm 0.6745\sigma$. The first interpretation of the results is that whenever a sample is chosen from a population, the chances are 68.26 out of 100 that its sample mean is within $\pm 1\sigma$ of the population mean.

2.4.1 Log-Normal Distribution

Not all quantities in the world have normal distributions. We find, for example, that concentrations of trace species in food and diet, in the atmosphere or other media are more often log-normally distributed than normal. Whenever the fluctuations of a quantity are comparable in magnitude to the mean value, there is a good chance that the distribution will be log-normal. In that situation, the normal distribution will predict significant probabilities for negative values, which make no physical sense. By contrast, negative values do not arise in log-normal distributions.

As shown in Figures 2.2a and b, a log-normal distribution simply means that, if one plots the probability *vs*. the logarithm of the quantity, the resulting distribution

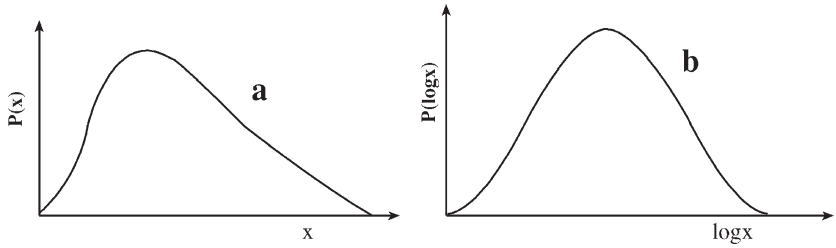


Figure 2.2 *(a) Normal distribution (b) Log-normal distribution*

is normal. In these cases, the calculation will involve the normal distribution function. Then Equation (2.8) will be in the form

$$y = \frac{1}{(x_i \sigma \sqrt{2\pi})} \exp\left[-\left(\log x_i - \log \bar{x} \right)^2 / 2\sigma^2 \right] \tag{2.9}$$

In general, if average and median are different, it is possible that distribution fits to log-normal distribution better than normal distribution, and geometric mean agrees better with median than mean.

For log-normal distributions, it is appropriate to calculate the geometric mean, x_g:

$$x_g = \sqrt[N]{\Pi x_i} \tag{2.10}$$

where Π indicates that one takes the product of the N values of x_i. Likewise the geometric standard deviation is given by

$$\sigma_g = 10^k \tag{2.11}$$

where

$$k = \left[\frac{\Sigma \log^2 x_i - N \log^2 \bar{x}_g}{N - 1} \right]^{1/2} \tag{2.12}$$

Note that σ_g is multiplicative: 66% of the points should fall within the range $\bar{x}_g/\sigma g$ and $\bar{x}_g \times \sigma$. Since, as seen in Figure 2.2b, the range below $\bar{x}_g$ is less than the range above $\bar{x}_g$, one should report the result with different negative and positive uncertainties, *e.g.* as $\bar{x}_{g\,-\sigma-}^{+\sigma+}$ rather than $\bar{x}_g \pm \sigma$.

2.4.2 Standard Deviation

The standard deviation s or σ is a good measure of deviation from the mean. It differs from mean deviation (see Section 2.3.5) by squaring the deviations from the mean instead of taking the absolute values as in mean deviation. The standard deviation of a population N, with value x_i and true value μ is

$$\sigma = \sqrt{\frac{\Sigma (x_i - \mu)^2}{N}} \tag{2.13}$$

Instead of a whole population or a greater sample from the population, if a sample is taken from the whole population sample (*e.g.* $N < 20$), the standard deviation of a sample, which is shown with s, is expressed as

$$s = \sqrt{\frac{\Sigma(x_i - \bar{x})^2}{N - 1}} \tag{2.14}$$

Since in most analytical experiments the number of measurements are <20, the calculated degrees of freedom will be decreased by 1, or N–1 is used instead of N in Equation (2.14).

When two or k sets of measurements have been combined into a single lot, it is possible to calculate the standard deviation of the total distribution from the standard deviation values of the two or more distributions. The pooled standard deviation, s_p, is given by

$$s_p = \sqrt{\frac{\Sigma(x_{i1} - \bar{x}_1)^2 + \Sigma(x_{i2} - \bar{x}_2)^2 + \cdots + \Sigma(x_{ik} - \bar{x}_k)^2}{(N_1 + N_2 + \cdots + N_k) - k}} \tag{2.15}$$

A very useful measure of precision is coefficient of variation, CV, or percent relative standard deviation, RSD:

$$\text{RSD} = \text{CV} = \frac{s}{\bar{x}} \times 100 \tag{2.16}$$

The CV gives the percentage and it is a ratio, which is independent of the units of measurement, therefore it is very useful in comparing the variability of a set of data measured under different conditions. A smaller s value or a leaner distribution, or even better way of expressing smaller RSD value is the indication of higher precision for a set.

2.5 Confidence Limit, Confidence Interval and Confidence Level

In most of the analyses, the data collected are limited with small number of measurements and the calculated mean $\bar{x}$, differ from the true mean, μ. The precision can be deducted from a series of replicate analyses and by calculating the mean. The next question is then how close is the calculated mean to the true value, which cannot be measured easily. The true mean can be derived from the measured mean within a degree of probability. This limit of probability is called the confidence limit. The interval defined by this limit is the confidence interval. The confidence limit, therefore, has to be calculated statistically from the measured mean and standard deviation within a confidence level as described below.

The normal curve in Figure 2.1 shows the distribution of measurements for a large number of data. The width of the curve is determined by σ, and true mean is close to the arithmetical mean within an error estimated from Equation (2.1). This is the sample data that can be used to determine a predicted range, confidence interval, for

Table 2.1 *Confidence level for z scores*

Confidence level	*z Score*
50	0.68
60	0.84
65	0.94
68	1.00
70	1.04
75	1.15
50	1.29
85	1.44
90	1.64
95	1.96
96	2.00
99	2.58
99.7	3.00
99.9	3.29

the true mean. It can only be stated that to a degree of certainty the population mean or true mean lies somewhere in that range. For example, as stated above, the true mean is in the range of $\pm 1\sigma$ with probability of 68.26%; in the range of $\pm 1.3490\sigma$ with a probability of 82.26%, *etc*. Therefore, different portions of areas under the normal curve can be related to a parameter, z values or z scores to make it possible to predict the range for true mean μ, for a selected degree of certainty. The probability of prediction is called the confidence level and the coefficient indicates the z scores. The values of z for different confidence levels are given in Table 2.1.

The relation between true mean, μ and sample mean, $\bar{x}$ will be

$$\mu = \bar{x} \pm \frac{z\sigma}{\sqrt{N}} \tag{2.17}$$

2.6 Student's *t* Distribution: Confidence Limit for Small Number of Measurements

When the numbers of data decrease below about 20, the normal curve can no longer be accurately used to describe the distribution of the sample mean. In this case, a different family of curves, which becomes broader with a decrease of sample numbers, is used. These curves are called t curves and show normal curve characteristics. The shape of any t curve depends on the degree of freedom (df), which is in most cases equal to the number of measurements N minus one (df $= N-1$). For large degrees of freedom, if $N > 20$, t curve becomes the normal curve and z scores can be used.

Table 2.2 shows the t scores for different confidence levels. The predicted range for the population means, μ, from standard deviation of s and mean, $\bar{x}$, will be

$$\mu = \bar{x} \pm \frac{ts}{\sqrt{N}} \tag{2.18}$$

Table 2.2 *t scores for various levels of confidence*

Degree of freedom	*Level of confidence (%)*				
	50	*90*	*95*	*99*	*99.9*
1	3.08	6.31	12.71	63.66	636.6
2	1.89	2.92	4.30	9.93	31.60
3	1.64	2.35	3.18	5.84	12.94
4	1.53	2.13	2.78	4.60	8.61
5	1.48	2.02	2.57	4.03	6.86
6	1.44	1.94	2.45	3.71	5.96
7	1.42	1.90	2.37	3.50	5.41
8	1.40	1.86	2.31	3.36	5.04
9	1.38	1.83	2.26	3.25	4.78
10	1.37	1.81	2.23	3.17	4.59
11	1.36	1.80	2.20	3.11	4.44
12	1.36	1.78	2.18	3.06	4.32
13	1.35	1.77	2.16	3.01	4.22
14	1.35	1.76	2.15	2.98	4.14
15	1.34	1.75	2.13	2.95	4.07
16	1.34	1.75	2.12	2.92	4.02
17	1.33	1.74	2.11	2.90	3.97
18	1.33	1.73	2.10	2.88	3.92
19	1.33	1.73	2.09	2.86	3.88
20	1.33	1.73	2.09	2.85	3.85
21	1.32	1.72	2.08	2.83	3.82
22	1.32	1.72	2.07	2.82	3.79
23	1.32	1.71	2.07	2.81	3.77
24	1.32	1.71	2.06	2.80	3.75
25	1.32	1.71	2.06	2.79	3.73
26	1.32	1.71	2.06	2.78	3.71
27	1.31	1.70	2.05	2.77	3.69
28	1.31	1.70	2.05	2.76	3.67
29	1.31	1.70	2.05	2.75	3.65
30	1.31	1.70	2.04	2.75	3.65
40	1.30	1.68	2.02	2.70	3.55
60	1.30	1.67	2.00	2.66	3.46
120	1.26	1.66	1.98	2.62	3.73
∞	1.28	1.65	1.96	2.58	3.29

2.7 Testing for Statistical Hypothesis

The above-discussed distributions can be applied to a number of experimental results in order to obtain more meaningful mean, compare experimental results, reject outliers, and compare standard deviations and other variables.

2.7.1 Comparison of Experimental Means with True Value or with Each Other: Student's *t* Test

An important statistical application is to estimate the agreement between experimental results with a true value or test result of the sample with standard sample. If

s is known from the earlier experiments, then confidence limits can be calculated for a given confidence level by

$$x_i = \mu \pm ts \tag{2.19}$$

Similarly, confidence limit for a mean value can be found for N experimental results by

$$\bar{x} = \mu \pm \frac{ts}{\sqrt{N}} \tag{2.20}$$

Two sets of experimental data obtained for the same sample by different methods or under various experimental conditions can statistically be compared within a confidence interval using z or t distribution tests depending on the size of data. If the replicate number of measurements are N_1 and N_2 for the first and the second experimental data with means of $\bar{x}_1$ and, $\bar{x}_2$ the true mean for each set will be

$$\mu_1 = \bar{x}_1 \pm \frac{ts_1}{\sqrt{N_1}} \tag{2.21}$$

$$\mu_2 = \bar{x}_2 \pm \frac{ts_2}{\sqrt{N_2}} \tag{2.22}$$

The null hypothesis can be applied to estimate the difference between the two means. This hypothesis states that there is no significant difference between two population means, and that the difference between sample means is a consequence of random errors only. Therefore, when $\mu_1 = \mu_2$ and pooled standard deviation, s_p, is used, the difference between means can be expressed as

$$|\bar{x}_1 - \bar{x}_2| = ts_p\sqrt{\frac{N_1 + N_2}{N_1 \times N_2}} \tag{2.23}$$

The interpretation of the data can be made by comparing the difference of means with quantity on the right-hand side of Equation (2.23) at the desired confidence level. The t value is taken at a selected confidence level of a degree of freedom, N_1+N_2-2. If $|\bar{x}_1 - \bar{x}_2| < ts_p\sqrt{\frac{N_1 + N_2}{N_1 \times N_2}}$, the difference between means is not significant. Otherwise a significant error is indicated at the given confidence level, which indicates the presence of a systematic error.

2.7.2 Comparison of Two Experimental Standard Deviations: The *F* Test

The F test can be used to compare standard deviations of two sets of data. In this case, the null hypothesis can be applied by assuming that the precision for both experimental data are identical. Therefore, the variance values, which are the square

of standard deviation, are compared. In fact, the critical value of F is the ratio of variances for two data sets,

$$F = (s_1/s_2)^2 \tag{2.24}$$

where the numbering is selected such that $s_1 > s_2$. The values of F for degree of freedoms $df_1 = N_1 - 1$ and $df_2 = N_2 - 1$ at a confidence level of 95% are given in Table 2.3.

The experimental F value calculated is compared with the value given in Table 2.3. If the calculated F value is greater than the tabulated value at selected confidence level, the difference between two data sets is significant.

2.8 Rejection of Outliers

In a series of measurements, certain results appear to be doubtful. Such results should not be rejected on subjective criteria; statistical tests must be employed. There are a number of tests which can be used for these extreme values.

2.8.1 Dixon's *Q* Criterion

This is a simple criterion for removing doubtful values. The results are first arranged in an increasing order. The difference between the doubtful value, x_d, which is either first or last in the series, and its neighbour, x_{d-1} is divided by the difference between the first and the last value, namely the range, giving the experimental Q ratio, Q_{exp}:

$$Q_{exp} = \frac{x_d - x_{d-1}}{x_n - x_1} \tag{2.25}$$

This is compared with the Q critical value, Q_{crit}, given in Table 2.4. If $Q_{exp} > Q_{crit}$ the doubtful value is rejected at that confidence level.

2.8.2 Student's *t* Criterion

In this case, the calculated value of t_{exp},

$$t_{exp} = \frac{x_d - \bar{x}}{s\sqrt{N/(N-1)}} \tag{2.26}$$

is compared with t_α values. In this case, $\bar{x}$ and s are the mean and the standard deviation for $N-1$ results other than the doubtful one. t_α is t parameter for $(N-1)-1 = N-2$ degrees of freedom. If the experimental value given in Equation (2.26) is higher than t_α, then x_d is rejected. The t_α values can be obtained from Table 2.2.

2.8.3 Gibbs's *R* Criterion

The R values given in Table 2.5 is compared with experimental $(x_d - \bar{x})/s$ value. As in the case of t criterion, $\bar{x}$ and s are the mean and the standard deviation for $N-1$

Table 2.3 *F values at 95% confidence level*

	ν_1																
ν_2	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*
1	18.5	19.0	19.2	19.2	19.3	19.3	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4
2	10.1	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.76	8.74	8.73	8.71	8.70	8.69	8.68
3	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.94	5.91	5.89	5.87	5.86	5.84	5.83
4	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.70	4.68	4.66	4.64	4.62	4.60	4.59
5	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.03	4.00	3.98	3.96	3.94	3.92	3.91
6	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.60	3.57	3.55	3.53	3.51	3.49	3.48
7	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.31	3.28	3.26	3.24	3.22	3.20	3.19
8	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.10	3.07	3.05	3.03	3.01	2.99	2.97
9	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.94	2.91	2.89	2.86	2.85	2.83	2.81
10	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.82	2.79	2.76	2.74	2.72	2.70	2.69
11	4.75	3.88	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.72	2.69	2.66	2.64	2.62	2.60	2.58
12	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.63	2.60	2.58	2.55	2.53	2.51	2.50
13	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.57	2.53	2.51	2.48	2.46	2.44	2.43
14	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.51	2.48	2.45	2.42	2.40	2.38	2.37
15	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.46	2.42	2.40	2.37	2.35	2.33	2.32
16	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45	2.41	2.38	2.35	2.33	2.31	2.29	2.27

Table 2.4 *Q_{crit} values at different confidence levels*

Number of observation	*90% CL*	*96% CL*	*99% CL*
3	0.94	0.98	0.99
4	0.76	0.85	0.93
5	0.64	0.73	0.82
6	0.56	0.64	0.74
7	0.51	0.59	0.68
8	0.47	0.54	0.63
9	0.44	0.51	0.60
10	0.41	0.48	0.57

Table 2.5 *Values of R at 95 and 99% confidence levels for Gibb's R criterion*

N	*95*	*99*
3	123	31.4
4	7.17	16.27
5	5.08	9.00
6	4.34	6.85
7	3.98	5.88
8	3.77	5.33
9	3.63	4.98
10	3.54	4.75
11	3.48	4.58
12	3.42	4.45
14	3.36	4.28
16	3.32	4.17
18	3.30	4.08
20	3.28	4.02
25	3.26	3.94

results excluding the doubtful one. As before, if $(x_d - \bar{x}_1)/s$ is larger than R value, x_d is rejected.

The following example explains how to use the above criterion for doubtful results.

Consider the series of 10 results obtained for the Fe values in mg/kg in a given total diet sample: 21, 21, 20, 21, 26, 19, 18, 17, 18 and 19. Any of the value should be rejected?

***The Q Criterion*:** The 26 mg kg^{-1} value could be an outlier or x_d value. At 95% confidence level for $N = 10$, Q_{crit} is 0.412. The Q_{exp} value is

$$\frac{26-21}{26-17} = 0.556$$

Since 0.556 is larger than 0.412, 26 mg kg^{-1} value should be rejected.

The t criterion: For the series of nine values (except 26), $\bar{x} = 19.3$ and $s = 1.5$. At 95% confidence level, for $10-2 = 8$ degrees of freedom, $t_\alpha = 2.31$. So from Equation (2.22),

$$t_{\text{exp}} = \frac{x_d - \bar{x}}{s\sqrt{N/(N-1)}} = \frac{26-19.3}{1.5\sqrt{10/(10-1)}} = 4.24$$

Therefore the value of 26 should be rejected.

The R criterion: Again for $N = 10$, at 95% confidence level, $R = 3.54$. Then $\frac{(x_d - \bar{x})}{s} = \frac{(26 - 19.3)}{1.5} = 4.46$. So the experimental result 26 should again be rejected.

As seen, all of the three criterions gave the same result, namely, the value of 26 should be rejected. On borderline cases, one may not obtain the same conclusion from the entire criterion. Then the experimenter should make the decision, or better take all the data for further calculations.

2.9 Linear Regression Analysis

In linear regression, the best-fitting line through a series of data points is drawn. These types of operations are very common for

(i) evaluation of the calibration functions of analytical systems and
(ii) finding linear relations among the variables in the multivariable systems.

As an example for (i), in most analyses, a calibration curve has to be constructed to predict the concentration of an unknown. In this case, standards containing known concentrations of the analyte are treated in the same way as the unknown sample. In order to draw the best-fitting line, some mathematical approximations are made. The simplest and most common treatment is the least-squares method. The uncertainties in regression operation are expressed statistically in terms of coefficient of correlation. The application of least-squares treatment to the calibration curve is based on the assumptions that the concentration of standards are known exactly, and a linear relation exist between the concentration of analyte and the measured variable.

As in the case of trace element analysis in food and diet, several elements are analysed in many samples and it is important to find the relation between these elements. For example, a linear relation is expected between the concentrations of Na and Cl in diet samples mostly due to the added salt, NaCl. Similarly many pairs of elements could show such linear relations, which could give rather important information about the sources of these elements.

In the least-squares method, the square of deviations for each point from the straight line is adjusted to be minimum. If the measured variable is y and the dependent variable x, the equation of best-fitting line will be

$$y = mx + b \tag{2.27}$$

where m is the slope of the line and b the intercept. Note that one should assign x to the more accurately known parameter and assume that all of the error is associated with y. In order to find the equation of a line, m and b have to be determined by the least-squares method, *i.e.* by minimizing the sum of the squares of the deviations of the points from the regression line.

The sum of the squares of the deviations, SSD, for each point from the line is

$$\mathrm{SSD} = \Sigma\,[y_\mathrm{i} - (mx_\mathrm{i} + b)]^2 \tag{2.28}$$

Taking the partial derivatives of this expression with respect to m and b, and equating to zero, the following equations can be obtained for n pairs of values:

$$nb + m\Sigma\, x_\mathrm{i} = \Sigma\, y_\mathrm{i} \tag{2.29}$$

$$b\,\Sigma\, x_\mathrm{i} + m\Sigma\, x_\mathrm{i}^2 = \Sigma\, x_\mathrm{i}\, y_\mathrm{i} \tag{2.30}$$

From the simultaneous solution of Equations (2.29) and (2.30), m and b can be found as

$$m = \frac{\Sigma x_\mathrm{i} y_\mathrm{i} - (\Sigma x_\mathrm{i} \Sigma y_\mathrm{i})/n}{\Sigma x_\mathrm{i}^2 - (\Sigma x_\mathrm{i})^2/n} \tag{2.31}$$

$$b = \frac{\Sigma y_\mathrm{i} - m\Sigma x_\mathrm{i}}{n} \tag{2.32}$$

One can also determine uncertainties of the parameters:

$$\sigma_{b^2} = \frac{\sigma^2 \Sigma x_\mathrm{i}^2}{\Delta} \sigma_{m^2} = \frac{n\sigma^2}{\Delta} \tag{2.33}$$

where

$$\sigma^2 = \Sigma\,[y_\mathrm{i} - (mx_\mathrm{i} + b)]^{2/\mathrm{n}-2} \tag{2.34}$$

$$\Delta = n\,\Sigma x_\mathrm{i}^2 - \Sigma x_\mathrm{i}^2 \tag{2.35}$$

After the linear regression curve is determined, the degree of relationship between the random variables (x_i, y_i) can be calculated. This value is called the correlation coefficient and is usually denoted by r. The formula for correlation coefficient is

$$r = \frac{m\sigma_x}{\sigma_y} = \frac{m\sqrt{\Sigma x_\mathrm{i}^2 - n(x)^2}}{\sqrt{\Sigma y_\mathrm{i}^2 - n(y)^2}} \tag{2.36}$$

The correlation coefficient r can have any value between +l, an exact positive correlation, and −l, a perfect negative correlation, and zero for no correlation. Fluctuations of y arise from two sources: variations of x with which y is correlated via the regression line, and fluctuations about the regression line, which may arise

from experimental error in the measurement of y values or from dependence on other parameters not included in the regression.

One important aspect of writing the expression for r as we have in the first part of Equation (2.36) is that r^2 represents the fraction of the variance of y, σ_y^2, that results from variance of x. The greater the slope of the regression line, and the greater the variance of x, σ_x^2, the greater the value of r will be, and, thus the fraction of σ_y^2 explained by variation of x. The portion of σ_y^2 accounted for by error or dependence on other parameters is $1-r^2$.

One must be careful about the correlation between two quantities. Correlation coefficients are rarely zero, as a small correlation nearly always arises just by chance. The smaller the value of n, the smaller is the given value of r. If there are just two points, one obviously obtains a perfect correlation. As in all statistical events, it is impossible to conclude that two parameters are absolutely correlated or not. Instead it is common to quote the probability $P(r,N)$ that the observed correlation arose purely by chance. As shown in Figure 2.3, if there are only 10 pairs of points, r must be >0.54 to reduce the probability of a random correlation to a value smaller than 10%. However, if $N = 50$ and $r = 0.54$, the chance that the correlation arose from random fluctuations drops to less than 0.1%. Usually, one should place little confidence in a correlation unless the P value is $<1\%$. In the literature, one may see such statements as: "the variables are correlated to the 1% confidence level", meaning that $P < 0.01$.

It is very difficult to establish cause–effect relationships from correlation. The fact that x correlates with y does not necessarily mean that x determines y or *vice versa*.

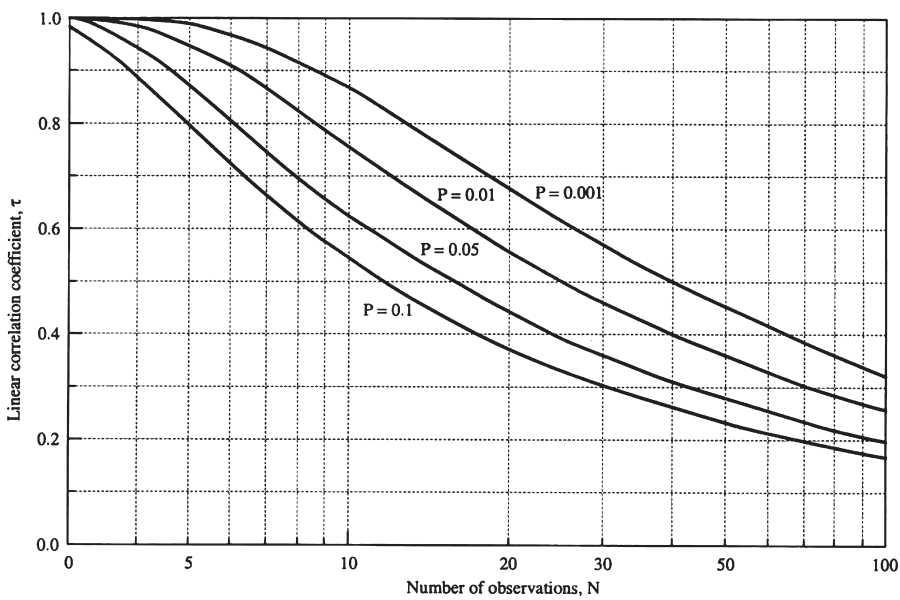


Figure 2.3 *The linear correlation coefficient r vs. the number of observations N and the corresponding probability P(r,N)*

Table 2.6 *Correlation coefficient between elements in a total diet*

	Na	Mg	CI	K	Ca	Cr	Mn	Fe	Zn	Se	Br	Rb	Sc	Co
Na	1													
Mg	0.61	1												
CI	0.99	0.58	1											
K	0.21	0.05	0.22	1										
Ca	0.21	−0.08	−0.04	0.63	1									
Cr	0.12	0.67	0.11	0.31	0.18	1								
Mn	0.4	0.55	0.38	−0.36	−0.49	0.29	1							
Fe	0.38	0.55	0.37	0.48	0.40	0.54	−0.0l	I						
Zn	0.14	−0.02	0.16	0.78	0.59	0.14	−0.54	0.66	1					
Se	0.23	−0.16	0.25	0.43	0.24	−0.07	−0.26	0.33	0.66	1				
Br	0.38	0.53	0.42	0.45	0.70	0.52	−0.04	0.70	0.46	0.04	1			
Rb	0.32	0.31	0.34	0.81	0.62	0.44	−0.31	0.90	0.90	0.41	0.72	1		
Sc	0.48	0.69	0.48	0.13	0.15	0.59	0.13	0.16	0.16	−0.01	0.58	0.42	1	
Co	0.47	0.84	0.45	0.16	−0.15	0.77	0.50	0.14	0.14	−0.06	0.45	0.45	0.67	1

The variations in both of them might be caused by some other variable z that is not included in the analysis. The concentrations of most elements in the total diet correlate significantly with the concentrations of most other elements. The main reason for this is that concentrations of most types of foods are all influenced strongly by few main staple foods like flour, vegetables and meat.

The correlation coefficients between the concentrations of elements give information about the sources of elements.[1] The correlation coefficients calculated for total diet samples[2] are given in Table 2.6. There is a strong correlation between sodium and chlorine; this is because the main source of these elements is the added salt, NaCl. There are also high correlation between Mg and Cr, Cl, Mn, Fe, Se and Co. Wolnik *et al*.[3] and Aras and Kumpulainen[4] showed that Mg, Mn, Zn, Se, Cr and V are the main minor and trace elements in wheat. Positive correlation between potassium and calcium and zinc indicate that they have a common source, namely vegetables.

2.9.1 Multiple Linear Regression

Often there is a possibility that a dependent variable is suspected of being dependent on several other variables. Multiple linear regressions can be used to test the dependence of one variable upon several others. For example, an element in total diet might be originating from different sources, such as water, several staple foods, spices.

Multiple linear regression assumes that one can express the independent variable

$$y = a_0 + a_1 x_1 + a_2 x_2 + \cdots + a_n x_n \tag{2.37}$$

The coefficients a_0, a_1, …, a_n are determined by minimizing the squares of the deviations of the y_i values from the plane defined by Equation (2.39). Determinations of the values as they involve some rather complex matrix methods are covered in Chapters 7 and 9 of Ref. 5.[5]

2.10 Receptor Models

The contribution of various sources to the total diet can be studied by using various approaches. Receptor modelling is one of the approaches to find the contribution of various sources. The basic idea of receptor modelling is to resolve the observed concentration patterns of trace elements at the receptor site. In our case, total diet is the receptor site, which has contributions from several possible sources such as various staple food inventories coupled with atmospheric particles, water and soil (Figure 2.4). Receptor modelling relates to the use of observed total diet concentrations for an array of chemical species (mostly trace elements) at a given time and site. This relation demonstrates the impacts of the several sources at that site, usually by some form of elemental ratios and multivariate techniques.

In general, receptor models use the chemical and physical characteristics of staple foods both to identify the presence of and to quantify source contributions to the receptor site. In this modelling,[6] the chemical and physical characteristics must be such that:

(i) they are present in different proportions in different sources and
(ii) these proportions remain relatively constant for each source type.

The above stated restrictions in applying receptor modelling do not cause serious problems in most cases, if suitable physical and chemical parameters are chosen. It is the reason why trace elements and stable inorganic ionic species rather than easily decomposing volatile organic compounds are widely used in receptor models.

Factor analysis (FA) and the chemical mass balance (CMB) are the two basic methods of receptor modelling. It is customary to run FA first to identify the sources

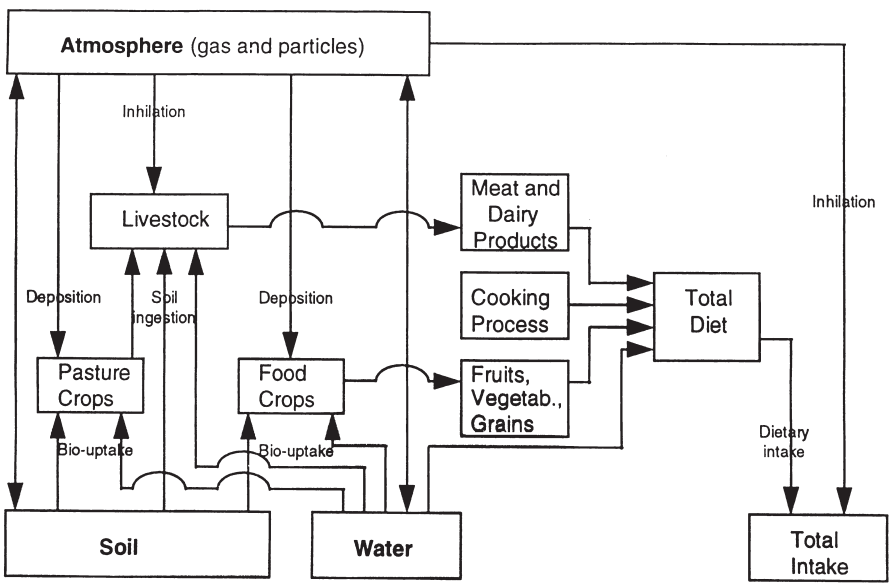


Figure 2.4 *Schematic representation of receptor modelling*

and later to apply CMB to quantify the contribution of these sources. In addition and prior to FA and CMB, several other statistical and mathematical approaches, such as linear regressions, trend analysis, trace element ratios together with the various enrichment factor calculations are applied statistical techniques employed to get a feeling about the nature of the full total diet data set.

2.10.1 Factor Analysis

Factor analysis is a most commonly used example of "multivariate analysis" which itself is a complex statistical technique for examining the large data sets to observe multiple correlation among the groups, which fluctuate together. In many research fields, a huge number of variables in different conditions are measured. The FA, a powerful statistical technique, is used to identify a relatively small number of factors that can be utilized to represent relationships among sets of many interrelated variables. The basic assumption of factor analysis is that underlying dimensions, or factors, can be used to explain complex phenomena, such as the sources of trace elements in total diet and daily intake of trace elements. One of the main steps of FA is to compute the linear correlation matrix for all normalized variables. Variables that do not appear to be related to other variables can be identified from the matrix and associated statistics.

Since FA is a purely statistical analysis technique, it requires no preliminary assumptions on the source profiles. During the calculations of the factor extraction step, the concentrations of elements in each sample are transformed into the normalized standard form given by

$$z_{ik} = \frac{x_{ik} - x_{\mathrm{i}}}{s_i} \tag{2.38}$$

where z_{ik} is the standardized value of the ith element for the kth sample
$i = 1,2,\ldots, n$ is the total number of parameters measured
$k = 1,2,\ldots, m$ is the total number of observations
$\bar{x}_{\mathrm{i}}$ = mean value of the ith element
s_i = standard deviation of the distribution of concentrations of the ith element
x_{ik} = concentration of ith element in the kth sample.

This kind of normalization takes equal weighting for each element, regardless of its average concentration. In factor analysis, the aim is to determine the minimum number of factors that can explain most of the common variance of the system. Owing to this normalization step, the quantitative information on each element is lost and factor analysis ends up with only the qualitative information.[7]

It is important to note that, for multivariate analysis, sufficient degrees of freedom should be available in the model. Thus, the data set employed must have many more observations than variables if stable results are to be derived.[8]

The primary objective of applying factor analysis is to derive a small number of components, which explain a maximum of the variance in the data. Initially the factor analysis results in as many components as there are original variables n. Usually, however, only a limited number of these uncorrelated components (*i.e.* 4–6) are

required to explain virtually all of the variance in data set of original intercorrelated variables. In order for this reduction in the size to be useful, the new variables (components) must have simple substantive interpretations. Empirically, it has been found that unrotated components are often not readily interpretable, since they attempt to explain all remaining variance in the data set.[9] This calculation results in a number of sources of variance being grouped together. For this reason, a limited number of components, which explain at least 80% of the total variance are usually subjected to rotation using a criteria such as VARIMAX.[10] After the VARIMAX rotation, the resulting components have been often found to be more representative of individual underlying sources of variation. This, in turn, results in more interpretable and useful components.

In the usual application of FA to any data set, it is desirable to have as many parameters measured in greater number of cases. However, in most of the experiments, almost every element measured could be missed in one sample or another, which made the factor score extraction an extremely restricted set. If one omits the whole sample in which an element used in FA was missing, half of the full data set could be wasted that makes no sense at all. A possible solution to this missing data problem is to fill the absent data with a suitable number. There are a number of methods to fill the missing data by using one of the following, such as:

(i) the most frequently observed value,
(ii) the mean value of the measured species and
(iii) a number found by the multi-linear correlation to the other elements (extracted by stepwise regression for all elements).

The last approach was found to be more suitable, *i.e.* to use Statgraphics software package to run stepwise regression for each missing element and find an equation as a result of correlation of each test element being dependent variable to other elements being the independent variables. The equation obtained for the test element Y is as follows:

$$Y = \text{Const.} + a\,[X_1] + b\,[X_2] + \cdots \tag{2.39}$$

where the constant number is the intercept and corresponds to the residue which is not explained by $[X_1]$, $[X_2]$ and $[X_n]$. The independent parameters X_1, X_2 and X_n were the measured concentrations in the samples in which the element Y was missing.

One of the major problems of factor analysis is the decision as to the number of factors to be retained. There are no simple rules applicable to each situation that has been advised to aid this choice. Each proposed rule has also generated criticism and counter-examples. It will often fall to the judgement of the investigator as to how many factors to keep. The purpose of factor analysis is to group the elements with the same variation into the same factor in a more understandable framework. The criteria that can then be applied is whether or not the factors can be interpreted in a way that aids in understanding of the system being studied. The factor analysis can be conducted using commercial statistical programs such as SPSS package or Statgraphics.

2.10.2 Chemical Mass Balance Method

Factor analysis and enrichment factors calculations provide primarily qualitative information about the possible sources whose contribution can only be determined by applying another chemical calculation method. In the CBM method, the other basic approach of receptor models, the concentration of an element i in the receptor site is assumed a linear combination of sources contributing to that concentration, and it is given as

$$c_i = m_j f_{ij} a_{ij} \tag{2.40}$$

where i is the type of the chemical species, j the source type, c_i the concentration of chemical species measured at a receptor site, m_j the mass concentration contributed during the sampling period, f_{ij} the mass fractionation term of element i emitted from the jth source and a_{ij} the fractionation term which accounts any loss or any gain, during any step of the cooking processes in the concentration of element i coming from jth source.

Since we assume no change for the concentration of any element in between source and the receptor site, a_{ij} values are usually set to 1 at the very beginning.

These equations have a unique solution only when the number of species is equal to or greater than the number of sources. Receptor modelling evaluation studies show that the greater the number of chemical species, the more precise the apportionment. Keeping these criteria, if one knows the composition of the total diet (*i.e.* c_i values) and source profiles of the dominant staple foods (*i.e.* m_j values), source contribution terms (*i.e.* f_{ij} values) can be determined by a least-squares fit to the observed concentrations of a set of elements called "marker elements". Marker elements are chosen among the strong indicators of staple foods and they are usually non-volatile elements, which can be measured, reliably in both total diet and the source samples.[2] This method also requires precision estimates for the c_i and f_{ij} values as model inputs.

The CMB model generally has the following assumptions:

(i) Compositions of sources are constant over the period of source sampling.
(ii) Chemical species do not react with each other, *i.e.* they add linearly.
(iii) All sources with a potential for significantly contributing to the receptor site have been identified.
(iv) The source compositions are linearly independent of each other.
(v) The number of sources or source categories is less than or equal to the number of chemical species measured at receptor site.
(vi) Measurement uncertainties are random, uncorrelated and normally distributed.

Although CMB studies provide valuable insight into the relative performances of several receptor models, certain limitations prevented a demonstration of the models' full capabilities. The major limitation is the lack of source profiles especially specific to the area where total diet is collected. They obtained most of the profiles from other places. Unfortunately, the true composition of a source is not usually measured but is approximated by employing a similar source type signature from the reference literature.

Up to this point the preliminary steps of source apportionment in total diet have been discussed. Some potential sources such as wheat and wheat products, vegetables, meat,

etc. have been identified as the contributors to the observed concentrations of the elements in total diet. Because of the limited number of the trace element results on various staple foods in the literature, much more work is needed for the receptor model-based source apportionment, which are the FA and CMB.

2.10.3 Enrichment Factors of the Elements

The qualitative approaches such as enrichment factors and elemental ratios are the preliminary steps of factor analysis and CMB based receptor modelling. The enrichment factor and the inter-element ratios are also the necessary steps to interpret the results obtained from the FA and CMB. A direct application of FA and CMB to a total diet data set may end up with uninterpretable results without these preliminary steps.

The enrichment factor model, is a double normalization technique, in which the elemental composition of the local total diet is compared with the elemental composition of the possible sources. That is,

$$(\mathrm{EF})\mathrm{source} = \frac{(C_i/C_n)\mathrm{sample}}{(C_i/C_n)\mathrm{source}} \tag{2.41}$$

where C_n is the concentration of normalizing element assumed to be uniquely characteristics of the source and C_i the concentration of an element whose enrichment is to be determined. In total diet studies, the source may be soil, flour, earth crust, vegetable, *etc*. According to above definition, if EF is around unity for the test element, one can assume that this element is entirely from the source used in calculations. Elements with enrichment factors greater than 2 are assumed to be due to the other sources rather than the source in question. The enrichment factor approach has been most useful when there has been a limited amount of information available. Enrichment factor approach cannot quantify a source's contribution, relies heavily on the assumed source composition and is not applicable to complex source mixtures where multiple sources, such as spices are contributing to the same element. Usually, in receptor modelling studies, the enrichment factor calculations and inter-element ratios are preliminary steps giving an insight about the characteristic of the trace element data set.

Aluminium, Sc or Fe are the common reference elements for the soil in $(\mathrm{EF})_{\mathrm{soil}}$ calculations. Elements, which have other sources as well as crustal dust, have enrichment factors higher than unity. The other sources, such as wheat products, vegetables and dairy products, contribute to the total diet.

Similarly, the enrichment of the elements with respect to wheat was examined in Turkey.[2] In this case the reference element was Mg and the source composition was taken from 7-year wheat averages.[4]

References

1. L.A. Currie, in *The Importance of Chemometrics in Biomedical Measurements*, K.S. Subramamanian, K. Okamoto and G.V. Iyengar (eds), Biomedical Trace Element Research, American College of Surgeons Symposium Series, 1991, 445.
2. N.K. Aras and I. Olmez, *Nutrition Suppl.*, 1995, **11**, 506.

3. K.A. Wolnik, F.L. Fricke, S.G. Capar, M.W. Meyer, R.D. Satzger, E. Bonnin and C.M. Gaston, *J. Agric. Food. Chem*., 1985, **33**, 807.
4. N.K. Aras and J. Kumpulainen, *Proceedings of the Technical Workshop on Trace Elements, Natural Antioxidants and Contaminants*, REU Technical Series, 1996, 49.
5. P.R. Bevington, *Data Reduction and Error Analysis for the Physical Sciences*, McGraw-Hill, New York, 1969.
6. G.E. Gordon, *Environ. Sci. Technol*., 1988, **22**, 1132.
7. R.L. Gorsuch, *Factor Analysis*, W.B. Saunders, Philadelphia, PA, 1974.
8. T.G. Dzubay, *Environ. Sci. Technol*., 1988, **22**, 46.
9. SPSS (Statistical Package for Social Sciences) Manual, SPSS Inc., Chicago, IL, 1990.
10. P. Koutrakis and J.D. Spengler, *Atmos. Environ*., 1987, **21**, 519.

CHAPTER 3

Trace Analysis

3.1 Scope of Trace Analysis

The meaning of *trace* is as arbitrary as the meaning of *small*. Generally accepted definitions for trace constituent and trace analysis are those referring to analyte concentrations lower than 100 mg kg^{-1}. Sometimes, the term *ultratrace* is used for analyte concentrations lower than 1 mg kg^{-1}. In our days, with novel advances in chemical analytical technology, pronunciation of femto or even attograms is not a rarity anymore; a recent study reported a detection limit (DL) of 10 pg L^{-1} (or 100 ag absolute) for Pb in human blood, by using laser excited atomic fluorescence spectrometry.[1] In order to deal with such low values, it is useful to know the commonly used metric prefixes given in Table 3.1.

The concentration levels for most of the essential elements in food are well established and it is not likely that lower DLs will be in demand for these analytes. However, there seems to be a relation between the legal restrictions and capabilities of analytical techniques employed for trace analysis. Better DLs obtained usually have resulted in lower legal limits for toxic constituents in food, environment and other health-related areas. In addition, the use of more potent analytical techniques in health-related research has helped to demonstrate the toxic effects of these elements at lower levels detected together with medical findings.

Therefore, the scope and capabilities of trace analysis is a continuously changing and improving field, where the demands and advances in analytical instrumentation direct and shape the present status.

Table 3.1 *Some metric prefixes*

Prefix	*Factor*	*Symbol*
milli	10^{-3}	m
micro	10^{-6}	μ
nano	10^{-9}	n
pico	10^{-12}	p
femto	10^{-15}	f
atto	10^{-18}	a
zepto	10^{-21}	z
yocto	10^{-24}	y

3.2 Methodology, Terms and Definitions

3.2.1 Sample, Analyte, Matrix and Blank

Sample is the name given to any material that contains *analyte*. *Analyte* is the target of the chemical determination, the species whose concentration is to be found. Sample may be in any physical form, solid, liquid or gas; it may be transformed from one form to another prior to the measurement step. Most analytical methods require solutions, but many food samples are solids; a dissolution step is necessary, transforming the sample from solid to liquid. Analyte may be an atom, molecule, ion or any other species to be determined. When the terminology is to be used correctly, the sample is to be *analyzed* and the analyte is to be *determined*.

The *matrix* is part of the sample that does not include the analyte. One should note that this definition is fulfilled only for trace analysis where the exclusion of analyte is negligible for the sample. For determination of Al in milk, it is possible to visualize a milk sample with no Al content; this would be the matrix for the mentioned analytical problem. However, if the purpose of an analysis is the determination of Ca in a marble sample; the analyte is included in the matrix since it is not meaningful to define a marble matrix with no Ca content.

During a chemical analysis, several steps of different operations such as filtering, heating, *etc*. and additions of reagents are usual and often unavoidable. These reagents may contain analyte as impurity. In addition, during the steps involved, some extraneous analyte may be added from the containers and other materials with which the sample is in contact. *Blank* is a material prepared and treated exactly as the sample except that it contains no intentionally added analyte. If the sample is liquid, pure water is used instead of sample to start preparing the blank. During the preparation of blank, all the steps are involved and all the reagents are added prior to the measurement step, exactly simulating the method applied to a sample. Measurement of blank may or may not show the presence of undesired, extraneous analyte signal, depending on the concentration level of analyte measured, purity level of reagents and lack or presence of contamination from the containers and environment. A repeatable and low *blank value* is desirable. The net analyte concentration is found after blank correction. In case of blank values too high to be accepted, the origin of contamination may be determined by preparing different blanks containing only one step or one reagent at a time. The reagent responsible for high contribution to blank value is replaced by the same chemical of higher purity in order to obtain a lower blank value. On the other hand, steps involved in a chemical analysis may cause the *loss of analyte*, as well as its contamination. Loss and contamination control in trace analysis is important (see Zief and Mitchell (1976) in the section on Further Reading).

3.2.2 Qualifications for a Trace Analysis Laboratory

In addition to the common safety and hygiene rules, there are certain qualifications to be implemented for a trace analysis laboratory. These are discussed below.

- *The laboratory* should have a clean environment. Ideally, a good temperature and humidity control in the laboratory must be provided. The laboratory should

receive the fresh and clean air required from a good quality air conditioning system. Some benches and hoods for sample preparation, transfer and treatment should be designed to have a positive pressure, so that no particles from outside should enter these areas, while clean and carefully filtered, particle-free air is supplied by high-quality fans. Laminar flow hoods with HEPA filters are commonly used in these critical areas. The use of *clean rooms* is becoming more and more common for trace analysis. Terms such as Class 10 or Class 100 are used for the quality of such rooms, corresponding to the presence of maximum of 10 or 100 particles with a diameter of 0.5 μm or more in 1 ft^3. Class 100, in SI units, would correspond to 3571 particles m^{-3}. Presently, the number of Class 10-clean rooms in world are < 10, while Class 100 is almost a must for trace analysis.[2]

- *Laboratory personnel*, the most important element in a trace element laboratory, must be dressed properly; clean shoes, gloves and a clean outfit, such as a white clean laboratory coat are mandatory. The possible contamination from body must be minimized by exposing as a smallest area as possible of hair, moustache, beard and flesh.
- *The vessels* used should be as clean as possible and should be inert for conditions of dissolution/decomposition process. Some laboratories have the tradition of using chromic acid solution for cleaning purposes; this reagent should not be used in a trace element food analysis laboratory. While there are elaborated cleaning procedures in the literature, a common and efficient method is soaking the vessels in (1+10) HNO_3 for few days, followed by several rinsings with pure water; once cleaned, the vessels should be dried in a clean environment and kept away from dust and contamination. Using multiple sealed plastic bags is an efficient way of storage.

  Often different vessels are used for sample treatment and storing the resulting solutions; the storage vessels should be kept tightly closed. If the solutions are left for long periods in the vessels, evaporation takes place even in closed vessels if not stoppered properly; further analyses on these samples cannot be trusted. In addition, such vessels will require more effort and more effective conditions for cleaning as their surface has been severely contaminated by dried constituents.
- *Water and other chemicals* should have the highest possible purity. Purity may be a relative concept in some cases. If a single element is to be determined, undetectably low levels of only this element in reagents may be sufficient. For simultaneous determination of several elements, the purity regarding only these analytes will be demanded. It is usually unlikely that the presence of interferant species will cause a problem, as their concentrations will be most likely below the level of interference; however, this possibility should be kept in mind as it may be important if very low-quality reagents with high-impurity levels are used. As the number of analyte elements increase, the requirements for purity will be more demanding; the conditions must conform to all of these elements; it becomes more difficult to obtain water and reagents to meet the requirements of a multielement analysis procedure. Most chemicals will have on their labels the results of an assay analysis for some impurities. While these figures are useful as a

preliminary guide, they should never be taken as an assurance of a specific purity. Careful blank measurements should be periodically carried out to check the purity for both the reagents and water. Water purification deserves a separate discussion that is given in the following paragraphs.

3.2.2.1 Water Purification

Pure water is an essential element of a chemical analysis laboratory. Since purity is a rather relative concept, each laboratory should try to produce water that has sufficient purity for the analyses on agenda. Most laboratories start this process by using tap water. Undesired impurities consist of cations, anions and uncharged molecules including organic compounds and free chlorine, Cl_2. Majority of elements that are subject of trace analysis are present as ions, while most metals are in cationic form, some elements are in anionic form, such as MnO_4^-, AsO_3^{3-} and CrO_4^{2-}. One criterion for water purity is low conductivity (or high resistivity); many manufacturers claim that the product has a resistivity as high as 18 MΩ. However, it must be remembered that this does not ensure the absence of many uncharged species, such as organic molecules. Another point worth noting is that high resistivity is indicated by sensors built *in* the water purification systems. As soon as the pure water is *out* of the purification system, carbon dioxide is rapidly dissolved in pure water, resulting in the production of bicarbonate and carbonate ions. Therefore, if a resistivity measurement is to be made on a pure water sample *outside* the purification system, values much lower than 18 MΩ will be observed. This result does not mean that the system is not efficiently purifying water, but is an indication of the difficulty for preservation of high resistivity in ambient air. In such a case, the water will still be pure enough to have a sufficiently low blank for many analyses, because the presence of dissolved carbon dioxide lowers resistivity, but does not introduce any contamination except CO_2, HCO_3^- and CO_3^{2-} species.

Elimination of organic components is necessary especially when chromatographic techniques are used, as some detectors are capable of detecting the organic impurities. Therefore, in order to avoid any unpleasant surprises, most chemists prefer to obtain water at the highest level of purity. The following principles of operation or their several combinations in a certain logical sequence are used in water purification.

Hardness removal. This is usually a preliminary step, rather than an effort to lower conductivity. Usually, columns containing inorganic ion exchangers in Na^+ form are used. Most of Ca^{2+} and Mg^{2+} are eliminated but an equivalent amount of Na^+ is introduced. The resulting resistivity is not much altered. However, the possibility of scaling in any further distillation process is minimized. Ion exchangers used in these systems can and should be regenerated by NaCl solution.

Distillation. Water is boiled, its vapour is transferred and is then condensed. Most of the ionic impurities are eliminated. Molecules more volatile than water are not eliminated by this method. Tap water should be introduced into a distiller after elimination of cations causing hardness, as explained above. Otherwise, scaling lowers the efficiency of heating elements and slows down the process of distillation; unit cost of distilled water increases as more electrical power will be consumed. Sometimes, oxidizing compounds such as $KMnO_4$ and Na_2CrO_7 are used in the boilers; therefore,

organic molecules are oxidized and decomposed. This system, although was very common in past, is not used very much since attention is required during operation. Another approach is *subboiling distillation*; water is evaporated by infrared radiation below its boiling point. The process is slower as compared to normal distillation by boiling, but the resulting purity is higher. This system is also successfully used in purification of mineral acids.

Ion exchange. Mostly polymeric cation and anion exchangers, which are in H^+ and OH^- forms, respectively, are used for removing ionic impurities in water. The relevant equations are given below for removal of cationic, M^+, and anionic, A^-, species. Similar equations can be written for ions of multiple charges:

$$R^-H^+ + M^+ \lambda R^- M^+ + H^+ \tag{3.1}$$

$$R^+OH^- + A^- \quad R^+A^- + OH^- \tag{3.2}$$

Water that is purified by ion exchange is called *deionized water* and the process is often called *deionization*. Ion-exchange resins have a capacity that will be exceeded after a certain degree of loading; at this point, the resin material should be renewed or regenerated if possible. Working principles of ion-exchange resins are illustrated in Figure 3.1. If separate columns are used in series, as shown in Figure 3.1A, the regeneration of each column material can be easily done by using HCl for cation and NaOH for anion resins. If a mixed bed is used, regeneration is not practical since the respective resin materials must first be separated; columns operating on the principle of

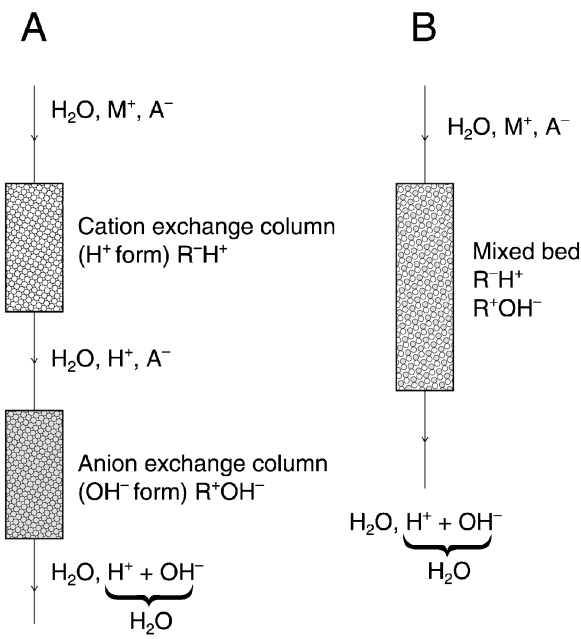


Figure 3.1 *Water purification by deionization using cation and anion exchangers. (A) sequential beds, (B) mixed bed*

mixed bed must therefore be renewed. However, in mixed beds, released H^+ and OH^- combine to form water; this constitutes another driving force for more efficient removal of cations and anions, resulting in an obvious advantage for using a mixed bed. All the commercial systems use mixed resins:

$$H^+ + OH^- \lambda H_2O,\ K = (K_w)^{-1} = 1.00 \times 10^{14} \tag{3.3}$$

Filtration. A column filled with a fine particular material that is not soluble in water is used to filter any solids present in water. Pure quartz is commonly used for this purpose. In addition, most commercial systems have a 0.22-μm filter at the end, just prior to the exit of pure water.

Activated carbon. Activated carbon reduces free Cl_2 to Cl^- that can be easily removed by ion exchange. In addition, many organic molecules or even transition metals are adsorbed on carbon surface. Such an element is usually placed before the ion exchangers.

Reverse osmosis. The principle of reverse osmosis has been used in purification of seawater to obtain water that is free of NaCl for common use. The same principle is also used in laboratory water purification systems. Pressure is applied on water, and molecules are forced to pass through a size-selective membrane; while water molecules are small enough to pass, larger ions or compounds are eliminated. In most commercial systems, chemical species larger than 100 Da are eliminated.

Electrodeionization. This is a rather novel approach to obtain water pure enough to be fed to high-cost–high-performance water purification systems. The mechanism is illustrated in Figure 3.2. In this system, membranes selectively permeable to cations or anions are used. An electrical field is applied to attract the cations to cathode and anions to anode. The pathway full of both cation- and anion-exchange resins

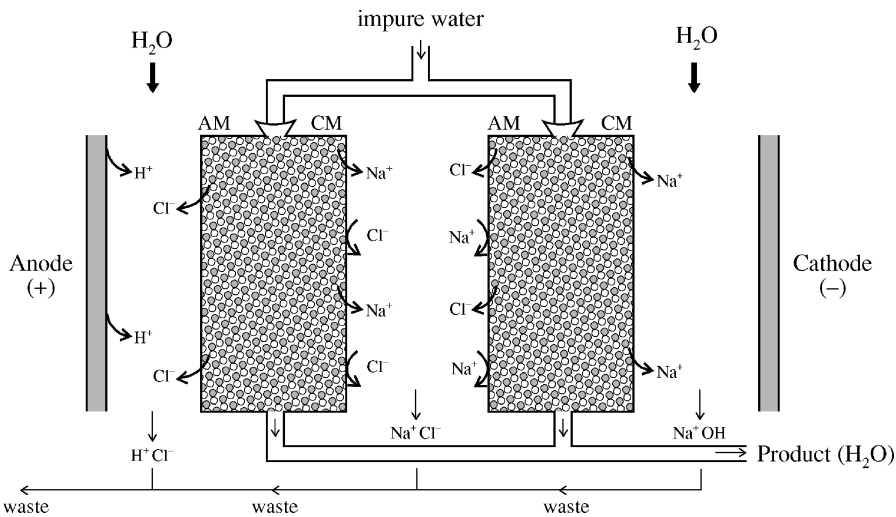


Figure 3.2 *Water purification by electrodeionization AM, anion permeable membrane; CM, cation permeable membrane (Adapted from Ref. 3 with permission form Millipore Corporation, France)*

are in contact with these membranes. The location of these membranes is designed in such a way that purified water and separated ionic contents are directed into different channels, called as 'product' and 'waste', respectively. The purified water can be stored until further use. The presence of ion-exchange resins facilitates ionic migration. It should be noted that since the ions are not permanently retained on the ion-exchange materials, these columns do not need to be regenerated.

Ultraviolet radiation. High-energy UV radiation damages bacteria, by either killing them or preventing their reproduction. Water is subjected to ultraviolet radiation as it passes through a channel that is radiated by an UV source lamp. This part is usually located towards the end of a purification train of several steps.

Typical water purification systems and general considerations. Water purification apparatuses are commercially available in the form of either separate modules or combined instruments, capable of functioning according to one or several of the principles discussed above. The following points should be considered in order to build and/or buy a water purification system.

Most elements in a water purification system require continuous attention and service. Ion-exchange columns should be checked for efficiency and replaced or regenerated when necessary. Distillation systems should be serviced and periodically cleaned from residues and scaling products that lower efficiency. While internal sensors such as a resistivity-measuring device sometimes indicate the purity, external checks should also be made. Any increase in water blanks should be carefully followed, and the performance of the water purification system should be kept at the desired level.

In most cases, tap water is the initial material to obtain pure water. Tap water contains impurities at high levels. The first step should be an inexpensive way of purification so that the product, water of intermediate purity, can be fed into more expensive, high-purity systems. This approach ensures a higher lifetime for more expensive components such as renewable cartridges.

Most important and abundant species causing water hardness and thus scaling are Ca^{2+} and Mg^{2+}. If any kind of distillation is to be used, these cations should be eliminated prior to being fed into a distiller; this can be done by water softening or electrodeionization. Theoretically, an electrodeionization system does not require regeneration of ion-exchange material and therefore is expected to have a longer lifetime.

Proper storage of purified water is as important as its production. Regarding material for storage containers, PTFE and some other fluorinated polymers are best but they are expensive. Many other polymeric materials such as high-density polyethylene are also suitable for production of storage containers. Period of storage should be kept to minimum. Glass should not be used for the storage of high-purity water; otherwise, high blanks for some elements such as boron should be expected.

Sequential systems for water purification can be used either as a continuous train or with some intermediate stages of storage, especially if the speeds of water production are not compatible for components.

Some systems for water purification are schematically given in Figure 3.3. The general strategy is first to prepare water of intermediate purity that can be fed into a high-purity water production system or used for analyses that do not demand very

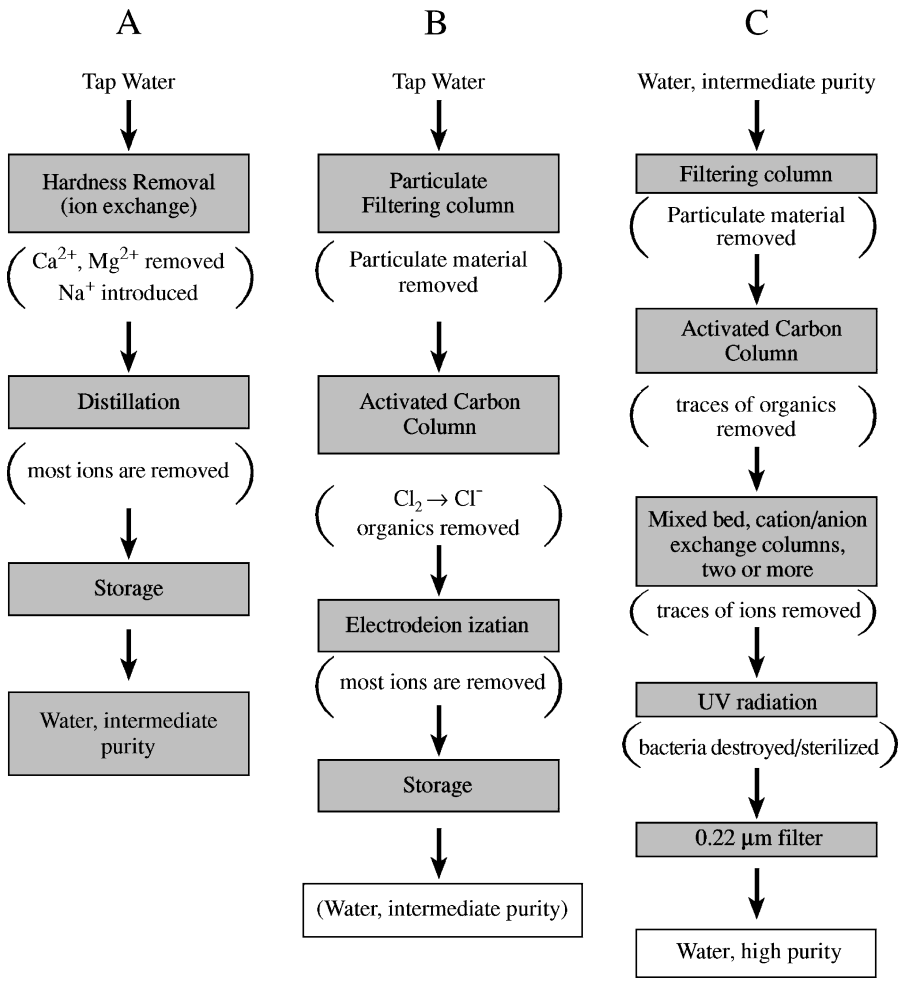


Figure 3.3 *Some systems for water purification in laboratory. (A) and (B) two approaches to produce water of intermediate purity, (C) a typical high-purity water production system*

high purity. At this stage proper storage has to be used. The final stage is used for production of high-purity water; the lifetime of components and their performance is highly improved if the preceding steps are properly designed and used.

3.2.3 Precision, Accuracy and Traceability

The terms *precision* and *accuracy* have been defined in Chapter 2. The most commonly used term for expressing precision is *relative standard deviation* (RSD). Very often, this value is multiplied by 100 to give %RSD, also termed as *coefficient of variation.* The terms *reproducibility* and *repeatability* have been suggested for precision

figures to be obtained between different laboratories and in the same laboratory, respectively.

Perhaps the most healthy and illustrative way to define a value obtained as a measure of precision would be a very detailed and clear description as to how it was obtained. In this respect, the number of data from which RSD value is obtained will have a certain importance. The nature and history of the samples providing input values for precision are also important. Let us consider a case where several flour samples have been obtained from the same mill. These were dissolved by acid treatment and digested to give a sample solution. The resulting sample solutions have been used to obtain analytical readings. Usually, the precision obtained by using several readings from the same aliquot will be better than the one obtained for solutions formed from different solid samples. This approach can be used to estimate the precision at each step of procedure.

Some researchers prefer to use the term *accuracy* in such a sense that both bias and precision are involved. In other words, the results will be rendered to be less accurate by degradation in precision as well as the presence of bias. The lack of accuracy is more difficult to assess as compared to deterioration in precision; a quality control (QC) using standard reference materials (SRMs) is needed. This subject will be handled in larger detail in Section 3.2.7.

Another term that becomes increasingly important is *traceability*. The results from an analytical chemistry laboratory must be accurate. These results often have significance for health, trade, environment or any other legally defined and controlled value system. Therefore, the accuracy of results must be proven in a continuous and legally recognized manner. The state of being accurate and all the documentation and effort spent for accuracy are defined by the term *traceability.* The concept of traceability will be handled in more detail in Section 3.2.7.

3.2.4 Calibration

Methods for chemical analysis are often classified as *absolute methods* and *comparative methods*. The former class includes volumetry, gravimetry and coulometry only; most of the methods applied for trace analysis are of the second kind, comparative in nature. Therefore, majority of the analytical techniques employed for trace analysis rely on a *calibration* for which the standards of known quantity or concentration are used to give the analytical signals to be used as inputs for a calibration. Normally, the confidence in the quantitative nature of standards is at such level that, for linear calibration, almost always the simplest linear regression methods are applied where the values on the x-axis, quantity or concentration of standards, are assumed to have no error; the values on the y-axis, analytical signals, are expected to have a random error pattern in the form of a Gaussian distribution. There are often real cases where these assumptions may not be justified. Nevertheless, the simplest linear regression is routinely applied in most cases. Therefore, all the necessary effort must be given to assess the accuracy for the analyte quantities or concentrations in standards.

Calibration plot is a graphical representation of analytical signals vs. standard quantity or concentration. The term *plot* is to be preferred instead of *line* or *curve*

which imply linearity or curvature since this property cannot be forecasted. The term *calibration line* can be used accurately where the analytical relation is linear; however, a non-linear plot can also be employed with a proper mathematical relation. Different analytical techniques may use various approaches for calibration. Once the linearity in a concentration range has been established, a single-point calibration check may be used to verify the assessed nature of the calibration line, in particular for automated systems that would require occasional recalibration during the prolonged analyses.

3.2.5 Analytical Figures of Merit

In order to describe the performance qualities of an analytical method, some measurable quantities are used; these are called *analytical figures of merit*. These numbers reveal the capabilities that can be reached by using an analytical method, such as the lowest concentration or amount measurable and the concentration values for which the method is applicable.

3.2.5.1 Detection Limit and Limit of Quantitation

One of the most important analytical figures of merit is *detection limit (DL)* or *limit of detection (LOD).* DL may be defined in terms of the analytical signal or concentration (or absolute amount) corresponding to this analytical signal. The conversion from signal to concentration units is simply done by using the calibration plot, which relates these two quantities. In either case, DL is the smallest quantity, which is statistically different than 0, the absence of the signal or analyte. Certainly, most analytical signals are meaningful only when compared to a *blank value.* Therefore, very often the DL refers to the smallest quantity, which is statistically different from the blank value. It should be remembered that several approaches are used to define and compute DL; therefore, the procedure used to calculate it should be clearly defined. It is not possible or practical to carry out meaningful analytical measurements at DL, because this value is inherently imprecise and involves an uncertainty of about 50% RSD. Useful measurements start at about 10 times the DL, a value that is commonly termed as *quantitation limit* (QL) or *limit of quantitation* (LOQ).

The following definitions are often used:

$$\mathrm{DL}_{3s} = 3s\,(1/m) \tag{3.4}$$

where s is the standard deviation of blank measurements and m the slope of the calibration plot.

When blank concentration value, C_{blank}, is significantly large, this definition may have the following form:

$$\mathrm{DL}_{3s} = 3s\,(1/m) + C_{\mathrm{blank}} \tag{3.5}$$

In this case, the concentration value of blank is a limiting factor for DL.

It must be stressed that particular analytical techniques may have different definitions of DL. Therefore, it is best to mention the approach and the formula used while reporting a DL, as well as all the conditions present in its determination.

3.2.5.2 *Analytical Range*

Analytical range is the part of the calibration plot, which is used for analysis, in order to obtain a required performance. For example, if the required precision is 2% RSD or better, a part of the non-linear portion of a calibration plot may also be included. On the other hand, the calibration plot may be totally curved and still may fulfil the requirements. Alternatively, a linear calibration plot may totally fail in providing the required precision, resulting in no usable range. Some of these cases are shown in Figure 3.4. Rapid and accurate computation facilities are present in most of the analytical instruments. However, even in our times, there seems to be a mental fixation for the absolute necessity of linearity in calibration.

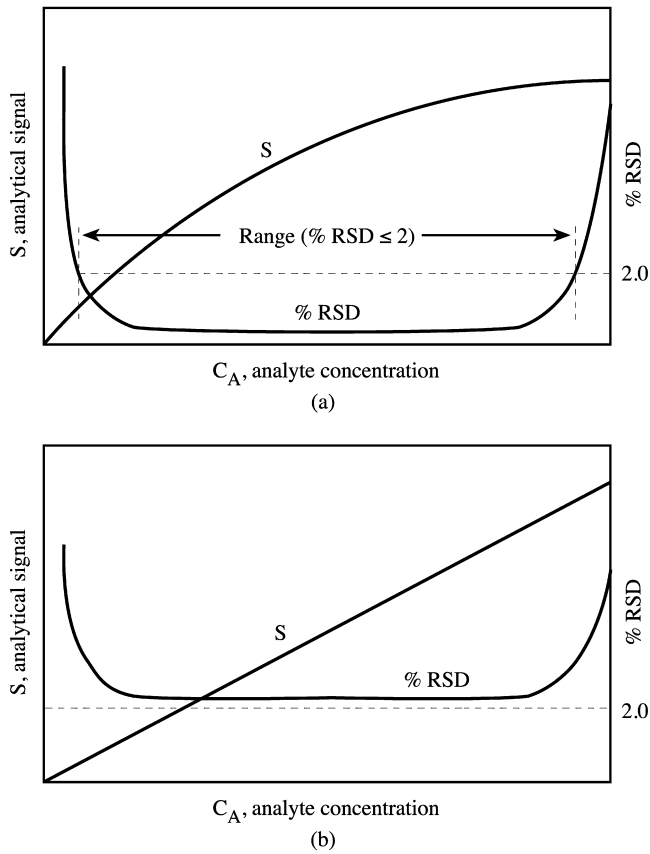


Figure 3.4 *Examples for analytical range and %RSD. Range for %RSD=2 or better (a) is given for a partly linear calibration plot; (b) does not exist for a linear calibration plot*

The *analytical range* starts at about LOQ and will end at the high concentration extreme where the required precision is not met anymore. The analytical range, sometimes termed as *linear range* or *dynamic range*, should be as large as possible; usually it is defined in terms of orders of magnitude. An analytical range of three orders of magnitude implies the coverage of 10^0 and 10^3 units (or 10^1 and 10^4, …) for analyte quantity or concentration in the same calibration plot. A large range has a significant practical value, especially when autosamplers are used. The samples having analyte concentrations lower than LOQ should be analysed by using another analytical approach or preconcentration techniques must be employed to bring the analyte concentration up into the analytical range. On the other hand, a large variety of concentrations can be handled in a large range. In cases where the analytical calibration has a rather small range, the samples with analyte concentrations higher than the upper limit will need to be rehandled and diluted; this will significantly reduce the effectiveness of using an autosampler. Recently, some commercial autosamplers used in atomic spectrometry are capable of properly diluting the sample until the analyte concentration falls in the range. The factor for this dilution is then used in computations.

Regarding the different concentrations each analyte will exhibit, the time saved by a large range will be more significant if a multi-element system is being used.

3.2.5.3 Sensitivity

Sensitivity is the degree of ability of a method to differentiate between two concentrations or amounts of analyte. When applied to comparative analytical methods, the *slope* of the calibration curve is used as the best measure of sensitivity, and is termed as *calibration sensitivity*. One should be reminded that for a linear calibration, the slope and thus the sensitivity is constant, where for a curved calibration, calibration sensitivity will be a function of analyte concentration. In other words, the precision is also very effective on the ability of differentiating between two close concentration values. Two calibration lines may have the same slope value, but if the one of them has better precision for both standard and sample measurements, the sensitivity will be superior as compared to the other line, because having a small standard deviation, closer analyte concentrations will be statistically different than each other when a test such as *Student's t* is applied (see Chapter 2). Some of the calibration slope values may depend on electronic amplification or attenuation of signal; this further complicates the definition of sensitivity in a universally comprehensible manner. The difficulties and confusions caused by both the electronic alterations of signal and precision problems could be solved by using another definition, *analytical sensitivity*, which is obtained by dividing the calibration slope value at any point by the standard deviation of analytical signal at that point. During any simple electronic alteration of signal such as amplification or attenuation, both signal and noise are subjected to the same change and therefore signal/noise (*S*/*N*) ratio remains constant. Signal refers to the instrumental response to analyte concentration where noise is the standard deviation of blank value (see Section 3.2.5.4). There are electronic hardware and software approaches to improve *S*/*N* ratio, but these subjects are beyond the scope of this chapter and can be found elsewhere.

$$\text{Calibration sensitivity} = m \tag{3.6}$$

where m is the slope of the calibration line

$$\text{Analytical sensitivity, } \gamma = m/s \tag{3.7}$$

where s is the standard deviation on the calibration line where the slope is measured.

By using analytical sensitivity values, laboratories and analysts at distant locations can have a more universal definition for their results.

Sometimes other definitions for the term sensitivity are employed. For example, in atomic absorption spectrometry, it has been customary to use "the concentration or amount which will cause a signal of 1% absorption (0.0044 absorbance)" as sensitivity; this is sometimes called as *reciprocal sensitivity* since it is inversely proportional to the slope of calibration line.

3.2.5.4 Signal to Noise Ratio

Signal, S, is the net response by an instrument induced ideally by the presence of analyte. Noise, N, is the standard deviation of many randomly distributed signals induced in the absence or presence of analyte. Sometimes, peak-to-peak noise is defined as shown in Figure 3.5. A ratio of these two quantities in a measurement is known as signal to noise (S/N) ratio. A high S/N is always desired. In this case, the uncertainty in S will be smaller and detection of smaller quantities can be detected.

3.2.5.5 Relations between Precision, Sensitivity, DL and S/N

Precision is most commonly expressed as RSD. Therefore, precision, analytical sensitivity, DL and S/N are all functions of s, standard deviation, for a defined set of measurements. If for N measurements, the standard deviation is s and the mean is $\bar{x}$, then

$$\text{RSD} = s/\bar{x} \tag{3.8}$$

Since for a set of measurements $S = \bar{x}$, and $N = s$, then

$$S/N = \bar{x}/s = 1/\text{RSD} \tag{3.9}$$

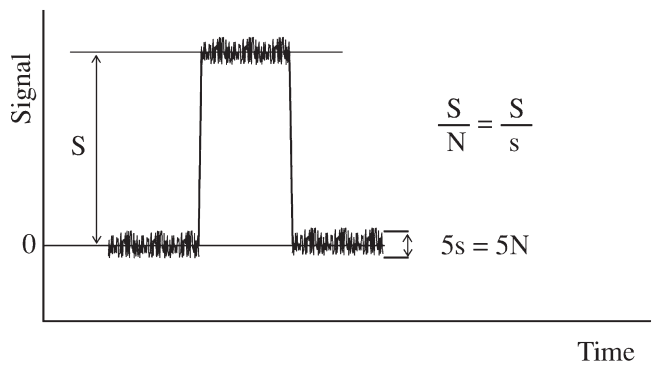


Figure 3.5 *Practical definition of signal (S), noise (N=s) and S/N*

On the other hand, analytical sensitivity is

$$\gamma = m/s, \textit{ i.e. Equation } (3.7)$$

If $m = \Delta\text{signal}/\Delta\text{concentration}$, for unit concentration,

$$m = S \text{ and } \gamma = m/s \text{ or } \gamma = S/N \tag{3.10}$$

It must also be remembered that $DL_{3s} = 3s\ (1/m)$ or proportional to s/m or $1/\gamma$.

Therefore, we can write the following relation:

$$S/N = 1/\text{RSD} \propto \gamma \propto 1/\text{DL} \tag{3.11}$$

The relation above emphasises the fact that the precision of a signal is very important. The requirements of high *S*/*N*, low RSD, high γ (sensitivity) and low DL all depend on a small standard deviation, the target of a high-quality signal and thus the result.

3.2.6 Selectivity and Interference

One of the most fundamental assumptions made regarding the calibration process is that the analytical signal varies with analyte concentration only. This is actually a common requirement for high *selectivity*, and this is the expected case for all the methods of chemical analysis. However, the analytical signal may be formed by the presence of *interferants* as well as *analytes*. A complete lack of *interferences* is a rather utopia case and at this point the term *specificity* can replace *selectivity*. In the real world of analysis, a method can have *high selectivity* at its best; it is very difficult, if not impossible, to justify the use of the term *specificity* in most cases.

A sample may consist of one or more analytes. The pre-treatment steps for these analytes may be the same such as in a simultaneous multi-element analysis. Alternatively, these steps may be different as in the cases of hydride generation, AAS determination of arsenic and cold vapour, AAS determination of mercury in the same sample; a pre-reduction by KI may be employed for arsenic. Therefore, the most useful definitions regarding the sample are made just prior to analytical measurement; this will include all the reagents, solvent, matrix modifiers and other necessary additives such as defoamers. In this last stage *analyte* is a constituent of the sample. The blanks must be prepared taking these differences in pre-analysis steps into consideration.

The *matrix* potentially includes *interferant(s)* that will affect the calibration plot in several ways. An interferant may compete with analyte in the formation of the analytical signal. For example, in most of the molecular absorption methods employed for element determination, a reagent that is selectively reacting with analyte is used to form an absorbing species. An interferant may competitively be reacting with this reagent if the *chemical selectivity* for analyte is not high; the product of this reaction will interfere if its spectral features are similar to the product originating from analyte; in other words, the *spectral selectivity* for analyte is not high enough. If these

conditions are met, interference will occur, resulting in the formation of extraneous analytical signal not related to analyte concentration in sample. On the other hand, an interferant may be totally inert from chemical point of view, but will cause an analytical signal, such as spectral that will cause interference. Most of these types of interferences do not depend on the presence of analyte and thus called *additive interferences*. In some cases, the interferant may alter the amount of the analyte that is available to cause the analytical signal; this may be an enhancement or depression which will result in an apparent increase or decrease in calibration slope, respectively. When the analyte is not present, this type of interference is absent; therefore, only the slope of the calibration is affected and the term *multiplicative interference* can be used. It is very often seen that an analytical signal may suffer both additive and multiplicative interferences simultaneously. The effects of additive and multiplicative interferences on a calibration line are shown in Figure 3.6.

These definitions for interferences lead to the conclusion that the additive interferences may be handled in the absence of analyte, but the multiplicative interferences should be handled in the presence of analyte.

Additive interferences may be characterized and handled by preparing samples containing all the matrix components but analyte; this is called a *matrix-matched blank*. Such a blank may be conveniently prepared if the matrix is well characterized such as a metal, an alloy or a well-defined synthetic material. The problem here is handled by either the direct use of a similar material that contains the analyte at a non-detectable level. Alternatively, reconstitution of the matrix from its well-defined components is attempted. This approach is called *matrix simulation*. After a successful matrix simulation, the blank may conveniently be spiked with known concentrations of analyte; a calibration line prepared by these samples can be used in correction of both the additive and multiplicative interferences. Unfortunately, this approach may often prove very difficult or impossible, since very high-purity components should be used in reconstitution and in some cases the matrix is too complex and cannot be simulated at all.

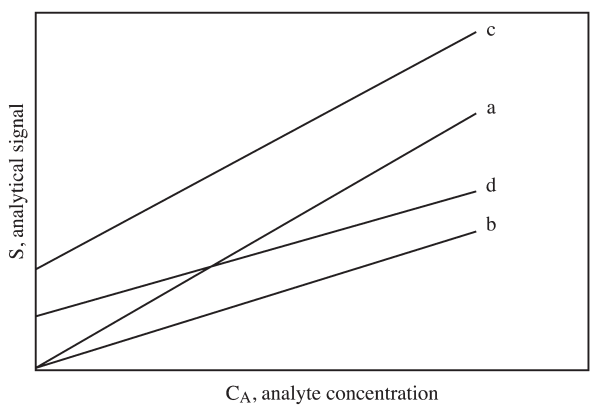


Figure 3.6 *Several kinds of errors on a linear calibration plot. (a) no interference, ideal plot; (b) multiplicative interference only; (c) additive interference only; (d) multiplicative and additive interferences*

In some cases, a blank is used to solve only a part of the additive interferences such as the analytical signals which may be caused by solvent, pH buffer and other reagents involved in analysis. In any case, blank measurement is made for correction purposes; the analyst must be aware of what is being corrected for. Some users would subtract the blank signal from the other readings; in this case, the calibration plot will tend to have a very small intercept. The better way is to use the blank signal as an input to calibration line where analyte concentration is 0 and then to use the calibration line with an intercept. This approach will provide a better visibility and thus appreciation of blank and more realistic evaluation of data is possible.

Multiplicative interferences can be handled in several ways. One of them is as described above matrix simulation and spiking with analyte. Especially for biological, clinical or food samples, this approach cannot be used as the matrices are too complex to be simulated. In this case, another approach may be used in which the sample itself is employed as the matrix for determining by adding successive analytes to the parallel portions of sample solutions and preparing a calibration plot from these spiked solutions. This approach is named as *method of standard additions*; *known additions* or *analyte additions* are also used. A calibration line prepared by the method of standard additions is shown in Figure 3.7. The intercept on the *x*-axis is used to calculate the analyte concentration after correction for dilution. The terms *endogenous* and *exogenous* are used to denote the analyte that is originally present in sample and spiked, respectively. While a calibration is prepared by standard additions, it would be very beneficial to construct another calibration plot at the same time by using standard solutions of analyte in solvent or in a simulated matrix. This is required because if the slopes are identical, the use of the standard additions is not needed, and the conventional calibration can safely be employed. It should be noted that for small number of samples, the use of standard additions would not make a large difference regarding the number of solutions to be prepared and measurements to be made; total analysis time is not affected much. However, as the number of samples increase, the extra load of work is obvious when standard additions technique is to be used. If the need for standard additions is justified, there may be another

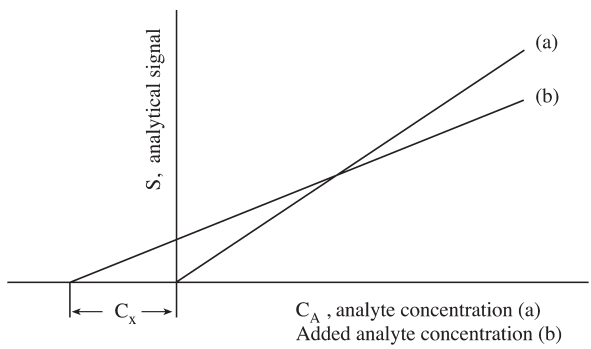


Figure 3.7 *Method of standard additions: (a) calibration plot for aqueous standards; (b) calibration plot for standard additions. C_x is the endogenous analyte concentration in spiked solution*

approach to minimize the amount of labour. If the information on the nature of the samples is available such that all the samples have similar matrices, a single calibration line by standard additions may be extrapolated to zero point and employed as a conventional calibration. In order to do this, a statistically sound number of randomly selected samples should be tested to see whether the slopes of the individual standard addition calibration lines have insignificant differences using statistical tests.

The observation of the following points is a minimum requirement in order to have a successful correction of multiplicative interferences by the method of standard additions:

(i) The analytical signals obtained must be in a linear range, so that extrapolation of line to obtain the intercept on the *x*-axis would not cause any error.
(ii) The endogenous and exogenous analyte species should be in the same form regarding the oxidation state, chemical environment, *etc*. Another way of expressing this fact is that the sensitivity for endogenous and exogenous analytes should be the same.

A large range of matrices are to be handled in food analysis. Therefore, the need for the use of standard additions should be frequently checked on new kinds of samples even when it is known that an aqueous calibration line has been known to be sufficient.

One vital point to be remembered is that the method of standard additions provides a correction for the multiplicative interferences at best only; the additive interferences cannot be eliminated by this approach. A rather useful demonstration of this point has been made by Welz.[4]

Internal standard method is mostly employed to improve the precision for a calibration and thus analytical determinations. Analytical signal may be affected in a random manner by analytical parameters such as weighing, diluting, flame or arc temperature. A second species, which is called as *internal standard*, is selected and added in a precisely known and constant amount or concentration to all the standards, blanks and samples. During the calibration, instead of analyte signal, a ratio of analyte signal to internal standard signal is used. The improvement in precision should be verified by comparing the respective calibration lines regarding the correlation coefficients (R) of both linear regression lines. It is expected that the use of internal standard will improve the quality of a linear fit, since the analytical signal caused by its presence should be a constant, and the ratio used should serve as a kind of normalization against fluctuations in the analytical measurement system.

The following points are to be observed and fulfilled for a successful application of internal standard method:

(i) The species chosen as internal standard should be present in all the standards, blanks and samples originally only at a level of concentration which is not detectable.
(ii) The analytical signals for the analyte and internal standard should be affected by the fluctuating analytical variables exactly in the same manner and proportions.

(iii) If there are any multiplicative interferences, their effect for analyte and internal standard should be identical. This condition may be more difficult to fulfil as compared to the others, since most of the multiplicative interferences are chemical in nature and therefore may be selective for analyte.
(iv) Internal standard should not cause any interference.

Internal standard approach is used best in minimizing the multiplicative interferences which function in a random manner.

In many cases, the absolute amount of the interferant is more effective to induce an observed interference than the interferant/analyte ratio. This behaviour allows one to dilute the sample to a point where the interference is not observed; naturally, at this point analyte concentration should be still higher than LOQ.

In some cases, the interference effect reaches to a saturation point; further increase in interferant concentration does not affect the analytical signal any more. If this is the case, it is possible to add more interferant to the sample until the analytical signal remains constant. At this point, the method of standard additions may be useful.

3.2.7 Legal Importance of Results, Traceability and Other Related Concepts

The term *traceability* was mentioned in Section 3.2.3. After covering other important definitions and concepts, it is more relevant to discuss this concept again. A laboratory needs to ascertain the quality of the results produced. There are now internationally used terms for control and assessment of quality in laboratories. *Quality control* refers to the process of investigating the accuracy of the results; *quality assurance* (QA) involves both QC and an effort to improve the accuracy. All the steps of analysis should be recorded in detail, so that whenever needed the relevant data such as sample preparation technique, instrumental parameters, standards used and the name of the person who actually carried out the analysis should be available. The results produced should be as accurate as possible with a well-defined *uncertainty*. It is increasingly accepted that legal analytical results without uncertainty are meaningless since no statistical work can be done on a number without uncertainty.

There is an internationally spent and shared effort to agree on some principles by which the analysis results are accepted by other parties. Analytical methods used must be validated. *Method validation* should be performed best by using SRMs. Analytical chemistry, after all, is a metrological science. There are national metrology institutes in many countries. These usually have connections with the other institutes that have internationally recognized authority. In this hierarchical order, there are well-defined laboratory accreditation procedures. The individual laboratories can obtain laboratory accreditation by applying to authorized national or international institutes following the required hierarchy. Once a laboratory earns laboratory accreditation from such an institute, the results produced in that laboratory will be recognized by other parties involved in the scheme under this accreditation process.

In the accreditation process, the quality of the laboratory infrastructure, instrumentation and personnel are questioned and examined. The candidate laboratory has to make the improvements as suggested. Several samples with known analyte

concentration are sent to the candidate laboratory. After proving an accepted performance, the laboratory accreditation is given; however, the control is continuous and the accreditation is valid only as long as the laboratory performance is acceptable by the criteria defined by the upper authority.

Laboratory accreditation is one of the most important elements of traceability. Probably, the most critical step in evaluation is that the laboratory must produce results within the accepted accuracy limits. This means that the results produced in the laboratory and the *known values* of the samples sent should not be significantly different. For this purpose, the samples with actual (natural) matrices and with known analyte concentrations should be prepared; these are named as *standard reference material* (SRM) or *certified reference material* (CRM). Internationally renowned research institutes[5–8] prepare homogenized samples with a natural matrix in rather large amounts, such as 30–100 kg. About 50–100 g portions are sent to laboratories selected all over the world. The analytes are pre-determined. After all the results are received, statistical tests are applied to reject some outliers and to determine the mean and standard deviation. Consequently, the *certified values* are listed with well-defined uncertainties such as s, $2s$, $3s$ or confidence limits using a given confidence level. The process of preparing an SRM is difficult and tedious; it may take as long as 2 years. At the end of the certification process, these SRM samples are made available to individuals who would like to test the accuracy of their own results.

It is generally suggested that for a set of samples to be analyzed in the same run, at least 5% of these samples must be SRM or well-characterized QC samples.

A very comprehensive compilation of SRMs available worldwide has been made by the International Atomic Energy Agency.[5] Several other national institutes[2,6,7] are also known to offer SRMs. European Union has a central facility for such purposes located in Belgium.[2] Among the other concerted efforts are EURACHEM[9] and ILAC[10] to help the analysts of the world for relevant, updated and useful information regarding accreditation and related concepts and principles.

An official definition of traceability has been made in the document called "ILAC-G2: 1994, Traceability of Measurements"[10]: "The term *traceability* means a process whereby the indication of a measuring system (or a material measure) can be compared with a national standard for the measurand in question in one or more stages". Important elements of traceability are listed below[9]:

- An unbroken chain of comparisons should exist between the individual result and a national or international standard.
- Each element in this unbroken chain must have well-defined uncertainties.
- Each step must be performed according to documented and generally acknowledged procedures; the results must be equally documented.
- The laboratories must supply evidence for their technical competence, such as laboratory accreditation.
- The results must be referenced to SI units; the appropriate standards must be primary standards for realization of SI units.
- Calibration and recalibrations of methods and instruments must be performed at required intervals, as often as possible.

Table 3.2 *Système international base units*

Quantity	*Unit*	*Symbol*
Length	Meter	m
Mass	Kilogram	kg
Time	Second	s
Electric current	Ampere	A
Temperature	Kelvin	K
Amount of substance	Mole	mol
Luminous intensity	Candela	candela

The Système International (SI) units are shown in Table 3.2.

Among the units shown in Table 3.2, *mol* is the most important one for chemical analysis. The mole is the amount of a substance consisting of elementary entities, as there are atoms in 0.012 kg of Carbon-12. The elementary entities may be atoms, molecules, ions, electrons, other particles or specified groups of such particles. One mole contains 6.022×10^{23} (Avogadro's number) entities as defined. While *mole* is the name of the concept, *mol* is the unit.

The efforts to have better definitions for concepts such as traceability, accreditation, *etc*. is continuous; although some references are given in this text, the reader should be aware of the dynamic character of these concepts, definitions and institutes.

References

1. E.P. Wagner II, B.W. Smith and J.D. Winefordner, *Anal. Chem*., 1996, **68**, 3199.
2. Institute for Reference Materials and Measurements, Belgium, http://www.irmm.jrc.be, 2002.
3. B.M. Stewart and D. Darbouret, *Int. Lab. News*, 1998, **28**, 25S.
4. B. Welz, *Fresenius Z. Anal. Chem.*, 1986, **325**, 95.
5. *Database of Natural Matrix Reference Materials*, A Compilation Prepared by the International Atomic Energy Agency (IAEA), July 2000, http://www.iaea.or.at/programmes/nahunet/e4/nmrm/index.htm
6. *Standard Reference Materials*, National Institute of Standards and Technology, USA, http://srmcatalog.nist.gov, 2005.
7. *Catalogue of Certified Reference Materials for Environmental Analysis*, National Research Council, Canada, http://www.ems.nrc.ca/env2000b.pdf
8. AGAL–National Analytical Reference Laboratory (NARL), Australia, http://www.agal.gov.au/NARL/index.html, 2005.
9. EURACHEM, *A Focus for Analytical Chemistry in Europe*, http://www.eurachem.bam.de, 2005.
10. ILAC Publications, International Laboratory Accreditation Cooperation, http://www.ilac.org, 2005.

Further Reading

1. J.D. Ingle Jr. and S.R. Crouch, *Spectrochemical Analysis*, Prentice-Hall, Englewood Cliff, NJ, 1988.
2. D.A. Skoog, F. James Holler and T.A. Nieman, *Principles of Instrumental Analysis*, 5th edn, Saunders College Publishing, Philadelphia, PA, 1998.
3. M. Zief and J.W. Mitchell, *Contamination Control in Trace Element Analysis*, *Chemical Analysis Series*, Vol 47, Wiley, New York, 1976.
4. E. Prichard, G.M. MacKay and J. Points (eds),*Trace Analysis: A Structured Approach to Obtaining Reliable Results*, The Royal Society of Chemistry, Cambridge, 1996.
5. M. Sargent and G. MacKay (eds), *Guidelines for Achieving Quality in Trace Analysis*, The Royal Society of Chemistry, Cambridge, 1995.

CHAPTER 4

Sampling and Sample Pre-treatment

Undoubtedly, sampling is the most critical phase of the entire analytical itinerary. Any factor affecting sample collection can be the source of errors that cannot be eliminated at a later stage. A recently published book by Crosby and Patel[1] concisely reviews general principles of good sampling practices for environmental, biological, food and other types of samples.

4.1 General Guidelines in Collection and Preparation of Staple Foods and Diets

All sample containers to be employed should be made of either polyethylene or polypropylene and preferably not used previously. However, if the plasticware containers have been in prior use causing trace elements contamination, a very rigorous cleansing procedure is mandatory as follows. After washing the containers using a clinical laboratory detergent, they should be left overnight in a 5% H_2SO_4 solution prepared from reagent grade concentrated H_2SO_4 and distilled water, followed by rinsing with double distilled water several times then oven drying at about 60 °C. Ensure that the oven has been washed carefully and lined with cellulose wadding. After acid-washing the interior surfaces, the containers should not be touched, sealed in a plastic bag. If in doubt any contamination that may arise from containers, it should be checked by using leached blank solution employing a proper procedure.

Rinse-cleaned equipment with 10% reagent grade sulfuric acid (only the parts which come into contact with the samples) then rinse with deionized water and finally with distilled water. Wrap knives in cellulose lining and dry them in oven. Cover cutting boards with cellulose lining and allow drying at room temperature. Only titanium-made corers and/or knives, 99.4% Ti, are acceptable for sampling of potatoes and the final meat samples.

The final pooling steps in preparing food and diet samples should be performed using polyethylene gloves; use of talc is to be avoided. A clean air hood, preferable class 100 should be used. The overall level of contaminants should be critically controlled by using proper blanks.

In processing the foods within groups as well as the final total daily diets, only homogenizers equipped with blades made of titanium or other material not causing trace elements contamination are acceptable.

In processing the main food groups that are predominantly composed of dry products, such as cereals and meat, tap water may be added for wetting. However, the total amount of tap water in the final diets should not exceed the average drinking water consumption level in each region. Additional distilled water may be used as required to facilitate proper homogenization.

For preliminary preparation of foods for the mixed diets, such as cutting off nonedible parts and transferring foods between containers, normal stainless steel-made kitchen utensils may be used. Afterwards, the food or diet should not be in contact with any materials that could cause trace elements contamination. Preferably, polethylene should be used for further handling and storage.

4.2 Sampling of Major Staple Foods

This is one of the most important steps in trace element analysis. The quality of the results depends on the care taken in sampling. This subject is thoroughly discussed by Crosby and Patel.[1] It is of utmost importance to design and facilitate sample collection protocol to ensure that the final pooled samples truly represent the entire regional or national production of the selected foods or consumption of daily diets.

For the sampling of a certain staple food, one has to take into account the main production areas and most important varieties for human consumption. Wholesale and retail marketing channels must be thoroughly known. In terms of animal products, variables such as animal breed and type(s) of fodder used should be additionally taken into account. Furthermore, seasonal variation definitely affects the origin of vegetables and fruits and has to be taken into account when purchasing foods from retail stores for representative total daily diets. Finally, the size and population density of the country in question set certain requirements to the sample collection in order to obtain representative samplers.

The following methods for collection and preparation of staple foods for chemical constituent determinations are presented using partly some of the recommendations mentioned in the Joint FAO/WHO Guidelines document.[2]

4.2.1 Wheat

The best way of collecting representative whole wheat samples is to make sampling arrangements with state granary stores and commercial mills. The samples obtained through these sources should represent the most important wheat-growing areas in a country, or a region. The samples should be taken from the “silo-samples” of each granary store. A silo-sample is a sample representing the contents of one silo and should be collected continuously during binning. One silo-sample should be about 3–5 kg in size.

Again it is very important to confirm that the grain to be sampled is all of domestic origin and intended for human consumption. Spring and winter wheat should be sampled separately. Thus, assuming that 15 mills or granaries are available for sampling,

the following sampling system is recommended: two spring and two winter wheat silo-samples of 3–5 kg each will be collected from each of the 15 mills or granaries resulting in a total of $(2 + 2) \times 15 = 60$ samples and weighing 200 kg in total.

A sub-sample of 100 g will be taken from each silo-sample and combined to represent 2 winter wheat and 2 spring wheat samples for each mill (60×100 g $= 6000$ g). A final sub-sample of 100 will be taken to represent each mill, totaling $15 \times 100 = 1500$ g. Silo-samples with obvious soil contamination will be discarded and replaced by new samples. These final samples (1–5 kg) will be carefully mixed in a glass or plastic-mixing chamber and approximately 500 g of each sample should be used for analysis.

4.2.2 Wheat Flour

The type of wheat flour, which is called baker's flour, should be collected; this product should contain a minimum 0.5% of residue after ashing. This figure is a measure of mineral content of flour. Samples should be collected from the leading commercial milling companies.

The following sampling example is for 5 mills: arrange 1 kg flour samples to be taken weekly during 8 successive weeks from all of the 5 mills. This will result in 40 units of 1 kg samples. Divide the samples randomly into four 10 sample sets and pool 100 g sub-samples within each set to make a 1 kg final sample. Mix these samples carefully and take 500 g sample for analysis.

4.2.3 Bread

In terms of ash content, the most commonly consumed type of white bread should be collected. It is suggested that regionally representative samples should be the target of sample collection. It is very important that an adequate number of the major bakeries are represented in the final samples of each region.

Similarly, the type of whole wheat bread that is consumed the most is to be collected. Whole rye bread should be collected in those countries where it has a significant role in the average bread consumption of the population. In some countries, mixed wheat and rye bread may be important cereal products, and consequently, the major types of such breads should be collected. It is recommended that five loafs totaling at least 2 kg should be collected and placed in clean polyethylene bags from each of the bakeries.

In the laboratory, combine the bread samples in such a way that half of each loaf is to be sliced for a pooled sample. Allow the slices of bread collected to dry over a period of time at room temperature, protected from dust under a loosely fitted polyethylene wrapping. When the slices are about half dry, they will be easier to homogenize in the food processor compared to a completely dry sample that is too hard for effective grinding. If the slices are fresh, on the other hand, it becomes too difficult to grind them because they will become mushy, which is difficult to work with. It is advisable to experiment first to determine the optimal sample moisture for homogenization. The samples should be sliced with a stainless-steel knife, however, the final homogenization in a food processor having Ti blades. After homogenization, the samples are either

freeze dried or oven dried in a cleaned and cellulose-lined oven at 60 °C. The final amount of sample material for analysis should be about 500 g dry weight per sample; at least four samples per region should be collected from several regions of the country.

4.2.4 Pasta

Collect all types of pasta samples from large wholesalers. Collect the best-selling pastas so that one sample represents each pasta's 10% unit of its total turnover. For example, if Company X sells 60% elbow, 30% spaghetti and 10% lasagna, then the amounts of samples to be collected are: 6 kg elbows, 3 kg spaghetti and 1 kg lasagna. Pool pasta samples in the laboratory are collected from different companies in a representative way so that the various types of pasta are pooled together to form regionally representative samples. Mix thoroughly these samples and homogenize a 4 kg mix sample using a food processor equipped with Ti blades. At least 4 pasta samples each weighing 0.5 kg dry weight should be taken for the final analysis.

In addition, the corresponding pasta flour samples should be collected. Pack pasta flours into polyethylene bags, then re-pack into a larger polyethylene bag to avoid contamination. The sample sizes are: consumer packages for macaroni and 1 kg sample for macaroni flours. Label the samples with tags containing sampling dates and, in terms of flours, the proportion of imported wheat, if known, and major production areas and crop year. The flour samples should be pooled to form regionally representative samples. Ti-blade food processors or acid-washed glass cylinders may be used for mixing the flour samples. Again, at least 4 flour samples each weighing 0.5 kg should be taken for final analysis.

4.2.5 Rice

Both white and converted rice samples should be collected. It is important to carefully determine the origin of the rice. Some European countries such as France, Italy and Spain have domestic rice production. The main emphasis is on the collection of domestic rice samples, if available, but for purposes of comparison it is advisable to collect imported rice from the major rice-producing countries. To guarantee adequate representativeness of samples, the best method is to collect samples from major wholesalers. Retail-sized packages should be collected over a period of time.

Pool the samples in the laboratory and carefully mix them in large, acid-washed polyethylene, polypropylene or glass cylinders. Use the pooling procedure suggested for wheat earlier in the text. The total amount of final samples should reflect the ratio of domestic to imported production. The final number of samples should be at least 4, representing the most important production area, or 4 imported samples if there is no domestic production. The final samples should be placed in polyethylene bags, with at least 0.5 kg per sample.

4.2.6 Potato

The most convenient way of collecting representative potato samples would be to make a sampling agreement with large potato wholesale companies or potato-processing

factories, which already have long-term agreements with the potato farmers to purchase their products. It is very important to confirm that the potato samples collected are varieties used and grown for human consumption only. Potatoes grown for the chemical industry or for fodder purposes are not acceptable.

A sampling system could easily be arranged so that the 2–3 kg samples taken would be a good representative of growing areas. Each final sample to be analyzed for trace elements should weigh a total of 50 kg (20×(2–3) kg sub-samples). After washing to remove soil, the two ends of each potato are removed and the potato flesh is sampled with a titanium corer (20 mm diameter). This would result in a sample of 2×5 cm and weighing 20 g. Each final sample would thus weigh approximately 12 kg fresh weight and provide about 1.5–2.0 kg dry material. Preferably, freeze drying should be used. If freeze drying is not possible, use oven drying overnight at 80 °C.

The individual 1.5–2.0 kg samples should then be ground in approximately 100 g portions each in a blender equipped with Ti blades. The ground portions will be combined and carefully mixed in a plastic or glass containers. Approximately 500 g of each final sample should be used for trace elements determination.

4.2.7 Meat

Tenderloin and pork shoulder are the best parts for the sample collection because they are not very expensive cuts, and thus will be easier to obtain at a reasonably low cost. In terms of broilers, the entire carcass will be collected. It is preferable that the samples be collected both in winter and in summer so as to reflect different feeding regimes.

Beef and pork samples should be collected from large slaughterhouses in such a way that each slaughterhouse collects the samples over a long period of time to guarantee adequate material representation of the samples, *e.g.* 30 large beef farms are represented by a slaughterhouse. Two 0.5 kg beef samples should be collected per beef farm in each slaughterhouse. The individual samples must represent different carcasses. Stainless-steel knives can be used for cutting the meat samples, as well as for the other meat samples.

Broilers should also be collected from large slaughterhouses similarly as for beef and pork samples, except that whole bird carcasses will be collected. For example, collect 40 broilers per slaughterhouse so that the material represents as many large broiler farms as possible.

Pack the samples immediately into polyethylene bags, coded with tags and then freeze. The tags should contain sampling dates and, if possible, the approximate locations of the meat producers (farms) to allow the exploration of the regional representation of the samples. The samples should be transported to the laboratory in a frozen state. They should be thawed and cut into 1 cm^3 pieces, transferred into frozen food containers and freeze dried. Only the edible parts will be utilized; discard skin or bones as well as subcutaneous fat. Poultry shears or stainless-steel knives may be used for cutting the meat samples.

For all sorts of meat samples, only a food processor equipped with Ti blades can be employed for the final sample homogenization. At least four final samples per one major production area are to be prepared for analysis.

4.2.8 Vegetables and Fruits

Collect vegetables during different seasons, *i.e.* in spring, summer and autumn so as to represent different growing conditions. If there are imported vegetables in the market, they should be collected separately and the country of origin of the produce should be established. Imported samples are usually available only during the winter season.

Taking cabbage as an example for collection of vegetables, cabbage samples should be obtained from a wholesale lot consisting of 10 heads; then 10 such wholesale containers should be randomly selected for sampling and one cabbage from each container is sampled. Then, the cabbages sampled as described above should be transferred into an empty container. The above principle should be used for all vegetables and fruits to ensure the necessary material representation of the samples. In terms of domestic and imported vegetables and fruits, the most commonly consumed varieties must be represented in the collection scheme according to their market share. The samples should be aggregated within varieties so that the final samples to be composed represent regional or national production or importation. Prepare four final samples per variety for analysis.

Here are some examples for sampling of carrot, cabbage, tomato, lettuce and apple. One could apply similar collection scheme for other vegetables and fruits.

Pre-wash carrot samples carefully using a scrub brush. Wash them in some place other than in the actual laboratory space where the final preparation of the samples will be performed. This is a very important precondition in order to avoid soil contamination that will totally destroy the possibility of obtaining meaningful results. Then rinse carrots with distilled water before peeling them. On a colourless polyethylene or Teflon cutting board, peel and cut them into small cubes, about 1 cm^3, using acid-washed knives. Mix the cubes and pack into acid-washed frozen-food containers, then freeze.

Remove the outermost injured leaves of cabbages and then rinse with distilled water. Cut cabbages into small pieces, mix and pack into frozen-food containers and then freeze.

Rinse tomatoes with distilled water and cut into quarters for the final sample. Transfer the sample into frozen-food containers and then freeze.

Remove non-edible parts of lettuce with a knife. Cut samples into small pieces with a knife and transfer into frozen-food containers and then freeze.

Freeze dry all the frozen vegetables, followed by homogenization using a food processor equipped with Ti blades.

Peel apples with acid-washed stainless-steel knives then cut them into 1 cm^3 pieces and transfer into frozen-food containers, freeze and freeze dry. Remove cores as well as stems.

4.2.9 Milk

The most practical way of collecting representative milk samples is to plan a sampling system with the leading dairy companies representing the most important milk production areas. As the trace element composition of milk is likely to be affected by the type of fodder the animals are fed, the seasonal variation may be important. Therefore, samples should be collected during both the grazing and indoor feeding

seasons. The samples representing the grazing season will be collected in September when the animals are still pasturing, and representing indoor feeding in February.

Whole milk, standardized approximately to 4% fat content and packaged into 0.5 L containers ready for consumers should be collected. Five packages are to be taken over a one week period and repeated every second week for a month totaling to 10 samples. The samples must be frozen immediately after collection. The packaged milk should be thawed at room temperature, then heated up to 40 °C and shaken to homogenize the fat. Aliquots of 50 mL are then transferred from each 0.5 L container into a 750 mL acid-washed polyethylene bottle to make one pooled 500 mL sample representing each dairy. The 24 units of 500 mL samples should be collected and then these will be divided into two 12-sample groups and the samples are pooled to make two final samples of 6 L each. These samples are then freeze dried, after which samples of approximately 750 g dry weight are obtained. Each will be used for trace element analysis. The winter samples will be prepared similarly, resulting in a total of 4 final samples.

4.2.10 Fresh Eggs

If the egg producers feed the birds differently in the summer than in the winter, then seasonal variation should be taken into consideration in the sampling. Otherwise, the samples can be collected during any season.

Samples should be collected from large wholesale companies. It is recommended that for every million kg of eggs produced, 5 egg producers should be chosen among the largest producers. Thus, if the packaged amount of eggs in that region or country is 6 million kg per year, then 30 individual producers' samples should be collected. The suitable size of the "producer sample" is 12 eggs (one dozen). Label the egg samples with tags describing sampling date, wholesale company, farmer and farm location. In the laboratory, pool the eggs in such a way that one egg per carton of 12 eggs is removed and pooled, according to region, into an acid-washed glass or plastic container.

The structure of the eggs is destroyed and mixed to avoid excessive foaming. Excessive mixing with stainless steel, egg beaters or whisks must be avoided. Place a 100 g sample each into frozen-food containers and freeze dry. Pool freeze dried samples to compose at least 4 final samples of 0.5 kg dry weight representing major production regions.

4.2.11 Other Staple Foods

Besides staple foods mentioned above, granulated potato and other staple foods important for a given country or region should also be collected for trace element analysis.

4.3 Collection of Diet Samples

It is most important to establish the level of average trace element intake of a person for many purposes. The data generated will thus be used

1. to compare the intakes with recommended values and
2. to be used by health authorities to assess dietary status and other public health measures.[3]

Therefore, it is important that the diets, which are to be analyzed, are representative of the country or region in question. To obtain nationally representative food consumption data, it is required that the sample amount should be sufficiently large and be selected by taking into consideration such variables as age, sex, race, income, region, urbanization and season. For example, in the United States, a recent national food consumption survey sampled over 20 000 persons for 3 days using 24 h recalls and food diaries. Although a smaller number of persons might have been adequate, the U.S. Food and Drug Administration pointed out that the totals should be a substantial segment of the total population.

4.3.1 Collection and Preparation of Foods for Composition of Representative Mixed Total Daily Diets, Market Basket Method

It is obvious that such large-scale food consumption survey is not an easy task. However, national food consumption or disappearance data are often available, which can be used to define the quantities of commonly consumed foods. It is therefore suggested that this approach be accepted as the principal method of preparing the diets to be analyzed for trace elements.

If data are available, diet composition should be based on the food consumption of the healthy adult population aged 25–40 years of each country or region. If these data are not available, adult population with a wider age range may be taken as a basis. However, in some cases the national food consumption data may not be very detailed and/or up to date. In such occasions or as an alternative to the market basket method, "duplicate portion method" can be employed.

If results of a recent national food consumption survey are available, they should be utilized, otherwise data on national food availability can be estimated from the OECD food consumption statistics,[4] which provides per capita consumption (in gram per day) of some 50 food commodities for most of the countries. The latest available statistics should be used.

Ideally, all foods whose daily consumption is 0.1 g or more should be included in the food list. In Western countries this would result in approximately 230 food commodities.[3] Household disappearance studies have shown that out of a total of 120 food items most commonly consumed, only 22 types of foodstuff were consumed in excess of 10 kg per year.[5] As representative purchasing and handling of 230 food items would be rather tedious, it is suggested that the following guidelines should be followed:

(a) All foods consumed in amounts of 1.0 g day^{-1} or higher should be included.
(b) If a food item or ingredient is consumed less than 1.0 g day^{-1}, it should be included only if it contains any of the 14 essential trace elements in concentration levels higher than 100 times that of a mean concentration level of the same elements in foods consumed in amounts of 100 g day^{-1} or more.

Food items which certainly would fall in the category (b) are many condiments, confectioneries, instant coffee and tea. A recent compilation of data on mineral element

concentration levels in 456 food commodities is available in order to define which food items should be included on the basis of category (b).[6]

In this connection, it should be mentioned that OECD Food Consumption Statistics[4] do not include food items such as coffee, tea and condiments, which are very rich in many trace elements. Moreover, only total refined sugar consumption is indicated, although much of it is consumed under the category of confectioneries, many of which again are very high in some of the trace elements. Therefore, it is proposed that the above-mentioned items be included in food lists to be developed.

The next step in the preparation of a food list is to group the foods according to major food types to be studied. Individual foods within these types should be ranked according to a decreasing order of daily consumption. It is recommended that foods be grouped into the following categories:

1. Cereals and bakery products
2. Dairy products (excluding butter and margarine)
3. Meat, poultry, eggs and meat products
4. Fish, crustaceans, mollusks and fish products
5. Potatoes and root vegetables
6. Leafy and legume vegetables, nuts and mushrooms
7. Fruits and berries
8. Non-alcoholic and alcoholic beverages, confectioneries, sugar and condiments
9. Food fats (butter, margarine, vegetable oils, lard).

The next step after grouping the foods according to major types would be to group food items under different categories, *e.g.* peaches as, fresh, frozen, canned, jammed and dried.

Unfortunately, as this type of aggregation is not largely applicable to national food disappearance data, the person responsible for the preparation of food lists should approximate the consumption of the food packed or processed differently.

The next step should be to aggregate foods that are similar in type but are consumed in small amounts, *e.g.* confectioneries. If the food consumption data are not very precise, the purchaser may decide which confectioneries ate to be included in the aggregate.

The shopping guide is developed from the food list. Special care must be exercised in the following points:

1. Purchasing foods of seasonal nature. Therefore, shopping should be done twice both in summer and winter
2. Similar foods should be purchased in such a way that they represent the most important manufacturers and brands
3. Packaging (fresh, canned, cartons)
4. Type of food transport and storage prior to kitchen preparation
5. The amount of food item to be purchased must be large enough to be representative and to allow losses during kitchen preparation.

Examples of shopping guides are available in Annex V a and V b in the FAO/WHO Guidelines.[1]

4.3.2 Collection of Food Samples

The purpose of the study should be defined very clearly. For example, if market basket diet study is applied to a whole country, then at least 4 cities representing different areas of the country should to be selected for purchasing of foods. In each city, all the foods are to be collected according to the same shopping guide. The first collection should take place in late summer or early fall, thus representing the summer season, and the second collection preferably in late winter to represent the winter season. During each collection (4 cities $\times$ 2 = 8) enough foods should be purchased to represent altogether 10 total daily diets in terms of amount of food, representing 80 total daily diets.

4.3.3 Duplicate Portion Technique

Duplicate portion technique is another way to collect the total diet samples.[7] The most convenient means to do this would be to collect 3–7 days duplicate diets from different population groups. One can select sampling sites, considering parameters such as socio-economical, cultural, regional variations and different age population groups. In all the cases, the subjects are not supposed to alter their eating habits during the collection period.

For a general survey of a country or a region, duplicate diet from major central hospitals may be most representative. Hospital food does not greatly differ from typical local food consumed in most countries and is thus supposed to be rather close to the national average.[8] Moreover, central hospitals have employed professional dietitians, therefore the sample and data collection will be done properly. Finally, from the financial point of view this method is attractive, as the hospitals may be willing to donate the diet samples provided that the research results are made available to the hospital authorities.

The easiest way to collect the duplicate diet will be to give each subject a 5-L and a 1-L pre-cleaned polyethylene container for food collection. Samples will be collected for three, preferably seven consecutive days from every subject. During meal time, while the serving is done, the amount of food equal to the amount consumed by the person will be placed on a separate plate. Then the duplicated food on the plate will be transferred to the 5-L polyethylene container with the utensils used by the subjects. The subjects should take the 1-L container to their work or wherever they go during the collection period. Everything the subjects had consumed during this period will be duplicated and collected. This will include all the meals and drinks. The inedible parts, skin of fruits, seeds, bones will not be collected, as these parts are not eaten by the subjects. Containers immediately should be transferred into a deep freezer.

4.3.4 Homogenization and Freeze Drying

Homogenization is one of the most important steps for the trace element analysis of the total diet samples. Since the collected samples consist of the various food items such as fats and vegetables, they must be homogenized with great care before

analysis. The samples should be homogenized with a homogenizer having Ti blades in order to prevent contamination. The homogenized samples are then freeze dried and powdered in an all Teflon cylindrical mill. Samples should be weighed before and after homogenization and freeze drying in order to find their water content.

Now the samples are ready for further handling as described in the following sections.

4.4 Sample Dissolution and Decomposition

The following analytical techniques will require normally a liquid sample in food analysis:

Spectrophotometry
Spectrofluorimetry
Flame emission spectrometry
Flame atomic absorption spectrometry (FAAS)
Electrothermal atomic absorption spectrometry (ETAAS)
Atomic fluorescence spectrometry
Inductively coupled plasma atomic emission spectrometry (ICP-AES)
Inductively coupled plasma mass spectrometry (ICP-MS)
Gas–liquid chromatography
High pressure liquid chromatography (HPLC)
Capillary electrophoresis (CE)
Total reflection X-ray fluorescence spectrometry

Therefore, whatever the initial state of the food sample is, in cases where one of the above techniques are to be used, the laboratory sample to be employed for the instrument must be a solution. The *chromatographic techniques* and *electrophoresis* are mentioned in relation to speciation analysis in food samples. There is extensive research regarding the analysis of slurries or solids directly by techniques based on atomic spectrometry; however, most of the routine applications for these methods currently are applied only to solutions.

On the other hand, normally solid samples are required for *neutron activation analysis* and *X-ray fluorescence spectrometry*.

The steps involved between taking a sample and performing the determination contribute significantly to overall error for the final result. The errors involved can be collected in two groups, namely sampling with sample handling and analysis. Then, s_t, s_s and s_a can be assigned as the standard deviations for total analytical process, sampling with sample handling and analysis, respectively.

$$s_t^2 = s_s^2 + s_a^2 \tag{4.1}$$

s_t and s_a can be found experimentally and s_s then can be calculated; overall results and replicate analysis for the same sample can be used to calculate s_t and s_a, respectively.

Producing a liquid sample involves several steps for solid samples. In some cases, even apparently liquid samples must be subjected to decomposition processes so that a uniform analyte species is obtained prior to analysis. For example, most beverages such as cola drinks or fruit juices may have to be digested, although in some cases

they can be directly introduced to a flame AAS system. On the other hand, a homogenized diet sample may contain analyte in diverse chemical and physical forms. In order to obtain a uniform analyte, preferably in solution form, a complete decomposition is often required.

It should be noted that the terms *dissolution* and *decomposition* have different meanings. *Dissolution* involves obtaining a liquid solution without any solid particles suspended or dispersed containing the analyte without any reference to its chemical composition; analyte may exist in one or more forms as the result of dissolution of a solid sample. In some cases, a mixture appearing as a solution to naked eye may contain particles with dimensions smaller than the shortest wavelength that can be seen by human eye. Such a mixture may contain analyte partly in these undissolved particles. If there is any doubt that such a colloid is the result of a dissolution process, ultracentrifuging the mixture will show whether solid particles are contained; these will be separated at the bottom of the centrifuging tube.

Decomposition, on the other hand, is a process in which significant chemical and structural alterations are imposed both on the analyte and the matrix. In this case, it is more likely that the analyte in the solution is in a different chemical form than it was in the solid sample. For any particular analysis, the analyst should decide whether dissolution or decomposition is required. Since some analytical techniques, such as AAS, plasma-AES and plasma-MS, are also able to contribute to sample decomposition, dissolution may be sufficient in many cases. Whether a digestion is needed or not can then be based on some preliminary analysis results on standard reference materials in order to see the effect of sample preparation on accuracy. Literature is sufficiently rich to have the proper guide on this matter regarding specific cases.

The purpose of analytical procedure also will be decisive on whether dissolution or digestion must be used. Decomposition alone or dissolution and further destructive analysis, such as in a flame, furnace or plasma, will be useful to obtain the *total element concentration* without any reference to its original distribution among chemical species. On the other hand, if the analytical procedure is to be used for *speciation analysis* (see Chapter 11), the original analyte species should remain intact prior to determination. In other words, in a solution ready for determination, all the analyte species should keep their original structures and concentration distribution as they had in the main sample. Therefore, with the ever-increasing demand and facilities regarding speciation analysis, a complete dissolution, which is also non-destructive regarding the analyte species, appears to be an important and delicate task as a part of sample treatment.

The following kinds of error are likely to be introduced during sample dissolution and/or decomposition:

Erroneous transfer of analyte to solution. This type of loss is most commonly caused by an incomplete dissolution of analyte species; if a total element determination is sought, a negative error, *i.e*. a low result will be obtained. On the other hand, in case of a speciation analysis, the process of dissolution should not alter the equilibrium among the analyte species; each form should be transferred to liquid sample preserving its original concentration. For example, if a fruit sample is treated with an oxidizing reagent, the concentration of species with different valencies may

be altered, resulting positive and negative errors for the species with high- and low-oxidation states, respectively.

Loss of analyte by volatilization. Heat is often used to dissolve the solid food sample using almost always an aqueous mixture of reagents. At elevated temperatures, some volatile forms of analyte might leave the contents of the mixture. In oxidizing medium, Cl_2, Br_2 and I_2 may form and be volatilized. Dissolution procedures with hot HCl solutions are known to cause the loss of some analytes such as $SbCl_3$, $AsCl_3$ and $HgCl_2$. Other similar types of losses are also possible. Therefore, compatibility of the dissolving matrix, sample and the level of temperature must be evaluated prior to dissolution step.

Analyte contamination by dissolution/decomposition reagents. Different mineral acids, solids or other reagents may be used to dissolve/decompose food samples. Especially in trace analysis, even very low concentrations of analyte present in these chemicals may cause significant contamination, as the mass ratio of reagent/sample is often as high as 10 or even 100. The purity of all the chemicals including the water is thus important. A golden rule of sample treatment is the use of minimum amount of reagents for lowest level of contamination.

Analyte contamination and take-up by vessel surfaces. A variety of vessel materials, such as glass, quartz, platinum, polytetrafluoroethylene (PTFE) and other polymeric materials can be used in dissolution/decomposition procedures. These containers, no matter how well cleaned between the uses, may keep some analyte species either on their surface or even as amalgamated in case of metal containers. During the subsequent use of these vessels, analyte species may contaminate the sample, causing a positive error. On the other hand, analyte species may also be removed from sample solution onto the walls of the vessel, resulting in negative errors. The contamination/take-up behaviour of vessels should often be controlled using blank dissolution/decomposition reagents that are to be analyzed before and after the treatment involving the vessel. PTFE as a vessel material has very good properties; nevertheless its unsuitability for F determination should not be forgotten.

After this preliminary information, we shall now discuss the techniques used for sample dissolution and decomposition. Before this section, however, it is useful to outline the general principles of common sense to be followed for choosing and applying these operations:

- Ideal sample treatment is no treatment; but this is seldom possible.
- Minimum amount of reagents should be used. This will minimize the contamination errors.
- Minimum number of steps should be used. All the errors, of loss and contamination, should then be minimized.
- If possible at all, the reagents employed during decomposition should be removed from the medium. In this case, the contamination errors are not removed; however, the sample solution will have a simpler matrix.
- Ideally, the resulting solution should have a matrix as simple as possible. Dissolution of the analyte is not sufficient. In particular for the samples of food and diet, the heavy organic matrix should be destroyed so that during the analysis step, the difficulties caused by the presence of the matrix will be minimized.

For example, the analysis of the liquid sample will then cause lower background absorption for atomic absorption spectrometry and lower total amount of dissolved solids for plasma spectrometry. In the presence of simple matrices, analysis with many spectrometric techniques will be easier, since potential interferants have been removed from the matrix. Most atomic spectrometric techniques are applied more easily if the matrix contains HNO_3; problems arise if HCl is present. Presence of H_2SO_4 or H_3PO_4 will shorten the lifetime of Ni cones for inductively coupled plasma-mass spectrometry.

4.4.1 Dry Ashing Techniques

Food and diet samples have heavy organic-matrix structures. Dry ashing involves the destruction of this matrix without the use of any solutions. Since most of the analytes are in very low concentrations, if the initial weight of the sample is higher, the final solution will contain a higher analyte concentration. Dry ashing technique has one advantage of using relatively high sample amounts, as compared to modern closed vessel, microwave-heating systems. Up to 1.0 g sample may be used for open or closed dry ashing procedures.

Open dry ashing simply involves placing the sample in an inert crucible and heating in a muffle furnace at 450–550 °C. Crucible material may be quartz, porcelain or platinum. The vessels are uncovered; however, the sample height in the vessel should be low as compared to the total height of vessel walls. Sometimes stubborn matrices such as oils and fats will require pre-treatment with an oxidizing acid such as HNO_3. *Ashing aids* may also be added during dry ashing; sulfuric acid or magnesium nitrate can be used for this purpose.

Several elements, such as Cd, Hg, Se, Zn and Pb, may be lost by volatilization at temperatures lower than 500 °C during open dry ashing. In addition to these elements, losses of Ag, As, Be, Cs, Ge, Li, Ni, Rb, Sb and Sn are also possible. The matrix properties will be effective on volatilization of analytes. Most elements have volatile chlorides. In addition, organic forms of Hg, Se and Cd can volatilize more easily as compared to their inorganic compounds.

Closed dry ashing is realized commonly in a *Schöniger flask*. Pure oxygen is fed into the closed system to facilitate the oxidation and the destruction of matrix. This application is rather rapid and easy, but there are risks of explosion.

Plasma ashing is another alternative for dry decomposition. In this technique, oxygen gas is activated by high-frequency induction, and introduced into the solid sample at about 120 °C. The possibility of contamination is minimized, but decomposition periods are rather long, special apparatus is required and the number of samples per batch is limited.

4.4.2 Wet Ashing Techniques

This group of techniques involves the handling of sample decomposition in several aqueous reagents containing mostly inorganic acids. The term *acid attack* may also be used for these procedures. Mineral acids provide an efficient way of solid-sample treatment. It must be remembered that minimum amounts of reagents must be used.

In some cases, the mixture of reagents and sample are kept in contact for a certain period of time at a certain temperature. Process of removing the analyte from its matrix leaving an undissolved part is called *leaching*. Since almost all food and diet materials are totally digested by the body, leaching alone is not commonly used in trace food analysis. There are cases, however, where leaching must be used, such as gums, which are chewed but not swallowed and not totally digested by the body. Sampling should simulate the manner the food is consumed. Samples of tea, for example, are to be leached in a way similar to its common use. If they are swallowed during consumption, dissolution and decomposition of food and diet materials must be complete and there should be no solid residue left.

Wet ashing is possible with both open and closed systems as will be discussed. Before discussing the details of these procedures, it is useful to have information on the reagents employed for these techniques.

A list of most commonly used mineral acid reagents and their properties are given in Table 4.1.

Some general comments for the use of these most commonly employed mineral acids are given in Table 4.2.

All the acids and the mixtures containing acids are hazardous. Special care must be exercised.

Several mixtures containing one or more acids and occasionally other oxidizing reagents are used for dissolution procedures. Different properties of reagents, such as oxidizing and complexing may be used together to enhance the power of dissolution and decomposing. Sometimes, reaction products of mixed reagents are effective. In some other cases, the power of a reagent is moderated by the other reagent in the mixture.

The ideal procedure of acid attack should leave no traces of dissolving reagents in the resulting solution; this is seldom possible if at all. Sometimes the mixture is heated to expel the unused reagent from the final solution, during this process the temperature of the mixture must be controlled to prevent any loss of analyte(s).

Some examples of these mixtures and the procedures mentioned above are given below.

Aqua regia. This reagent is prepared by mixing 3 parts of HNO_3 and 1 part of HCl, by volume of their concentrated aqueous solutions. *Aqua regia* is a very potent mixture; oxidizing power of HNO_3 and complexing (Cl^-) ability of HCl function together. Reactive products such as Cl_2 and NOCl are formed; these have oxidation

Table 4.1 *Aqueous mineral acids and their properties*

Property	*HCl*	*HNO_3*	*H_2SO_4*	*$HClO_4$*	*HF*
Molecular weight (Da)	36.46	63.01	98.08	100.46	20.01
Weight percent[a]	36.5–38	65–70	95–98	60–70	48–51
Specific gravity[a]	1.18	1.38	1.84	1.66	1.17
Molarity[a]	12.0	14.2	18.0	9.9	28.9
Boiling Point[a] (°C)	110	122	338	203	112

[a] For the laboratory use, these reagents are available as their concentrated aqueous solutions. The indicated properties may vary slightly from batch to batch and are given for the aqueous reagents.

Table 4.2 *General comments on the commonly used mineral acids*

Acid reagent	*Comments*
Hydrochloric acid, HCl	A weak reducing agent; seldom used for organic matter. Dissolves carbonates, some oxides, some sulfides and phosphates
Nitric acid, HNO_3	An oxidizing agent; forms soluble nitrate salts. Often used for organic matter; also efficient for metals, alloys and inorganic matter
Sulfuric acid, H_2SO_4	Oxidizing when used hot; often employed with HNO_3. Since its aqueous mixture can be elevated to relatively high temperatures, H_2SO_4 can release volatile products, excess acids such as HF and HNO_3. Commonly used for organic and inorganic samples
Perchloric acid, $HClO_4$	A strong oxidant especially to be used for organic matter. Its oxidizing power is elevated when hot. Its direct use may cause *explosions*, care is required.[9] A pre-treatment with HNO_3, followed by addition of $HClO_4$ is a common and safe procedure. Special hoods must be used with this acid
Hydrofluoric acid, HF	Used to dissolve materials containing SiO_2, such as glass, quartz, ceramics, *etc*. Volatile SiF_4 is formed and some Si is lost in open systems. Since glass or quartz cannot be used as the container material, Pt or hard plastics should be employed

power higher than the either starting acid. The mixture dissolves most metals, alloys, sulfides and some other ores. *Aqua regia* is the only solution that can dissolve Au, Pd and Pt.

Mixtures of ($HF + HNO_3$), ($HF + HClO_4$) or ($HF + H_2SO_4$). In these mixtures, HF acts as an agent to dissolve siliceous materials by forming SiF_4(g) and SiF_6^{2-}(aq) while the other acid functions as an oxidizing agent. In case of the mixtures containing $HClO_4$ or H_2SO_4, the resulting solution is heated to remove traces of HF that is the most volatile acid in the medium. The presence of the other less volatile acid assures that the temperature will not be excessively elevated as long as some liquid remains. In some cases, the mixture is heated to dryness. The residue is usually dissolved in dilute HNO_3.

Mixture of HNO_3 and $HClO_4$. This is a mixture that is often used for organic samples such as food and diet. The sample is first treated with HNO_3; the easily oxidized components are decomposed. $HClO_4$ is then added to decompose the rest of the components, which are more difficult to oxidize.

Mixture of HNO_3 and H_2O_2. This mixture has two oxidizing reagents working together. Instead of H_2O_2, other oxidants such as Br_2 or $KClO_3$ may also be used; these can be used with other acid mixtures as well.

Lefort aqua regia (or inverted aqua regia). This is a mixture of 1 part of HCl + 3 parts of HNO_3 by volumes of concentrated reagents. Cl_2 and NOCl, the potent products of aqua regia, are also formed in this mixture.

Some complexing agents such as citrate and tartrate anions can be added to a mixture to keep some metal ions in complexed form soluble in the medium. Inert electrolytes are sometimes added to increase the boiling point of the respective acid, such as KCl for HCl and Na_2SO_4 for H_2SO_4.

There are two main procedures for acid-attack technique. These are *open digestion* and *closed digestion.*

4.4.2.1 Open Wet Digestion

The mixtures of sample and reagent acid(s) can be heated openly using *hot plates*, as these are much safer as compared to Bunsen burners that once used to be the main tool for heating in laboratory. The container material must not be attacked by the contents at high temperatures. Borosilicate glass can be used for most purposes; however, for trace analysis hard plastics or PTFE is a better choice. Most of the plastic materials will have a rather low temperature limit, PTFE can be heated up to around 260 °C. When HF is included in the digestion procedure, glass or silica cannot be used. In this case, PTFE can be employed if temperature is to be kept below 260 °C; a Pt crucible or container should be used for higher temperatures.

Aluminium-block heaters may also be used to contain a rather large number of borosilicate glass tubes for sample digestion by acid attack. These devices have cavities to be filled by glass tubes, temperature control is provided by a simple electronic system using thermocouples; duration of heating may also be fixed and thus temperature programmes can be applied. Long-neck glass tubes are used where only the bottom part of these containers are heated by the block heater; therefore, the cool upper part functions as a refluxing system. These heating devices are ideal when evaporation of the tube content is not desirable.

During open-digestion procedures, maximum care must be exercised to prevent spills, violent boiling of sample solution and other possible accidents. In some occasions, *evaporation to dryness* is required. Sometimes extra additions of the reagent(s) are made. Open-digestion methods have been mostly replaced by close-digestion approaches by which the obvious disadvantage of the former, loss and contamination, are minimized. Especially when the concentration level of analyte is low, ppm or less, open digestion should be avoided.

Even when a smooth digestion is accomplished by open digestion, the loss of some volatile species is inevitable. In hot HCl solutions, several volatile metal chlorides such as those of As, Hg, Sn, Sb, Ge, Se and Te may be removed from the mixture. Use of HF will cause the loss of fluorides of As, Ti and Ta as well as SiF_4 and BF_3. In a reducing medium, some elements may be volatilized in the form of hydrides, such as PbH_4, SeH_2, AsH_3 and SbH_3. Open-digestion systems may cause other losses depending on the composition of sample and reagents. For example, in a hot mixture containing Cl^- and H_2SO_4, Cr is oxidized to Cr(VI) by sulfuric acid and the volatile compound CrO_2Cl_2 will be removed from the medium. The presence of chloride in other mineral acids may cause volatilization of Bi, Mn, Mo, Tl and V.

4.4.2.2 Closed Wet Digestion

In this approach, the reactions for dissolution–decomposition take place in a closed vessel where the free expansion and evaporation of the contents is prevented. Closed digestion vessels, also often called as *digestion bombs*, are much safer than their open counterparts. The sample and the digestion reagents are placed in a thick-walled PTFE beaker with a snugly fitting lid. It is customary to wet a powdered sample with a few drops of pure water before it comes into contact with any other reagent. This container is placed in a jacket to assure safe sealing. First models of digestion bombs were heated in conventional ovens. In this case, the bomb jacket is mostly made from steel. The heat emanated by oven is transferred to steel jacket, then to PTFE vessel and finally to sample digestion mixture.

Digestion bombs can be heated above the boiling point of the liquid contained, because elevated pressure prevents boiling. Therefore, higher temperatures can be used as compared to an open digestion where the temperature is limited by boiling point as long as some solution exists. Because of this kinetic advantage, dissolution–decomposition takes place faster in digestion bombs. On the other hand, the use of concentrated H_2SO_4 in PTFE vessels requires a good temperature monitoring and control, as the boiling point of the liquid reagent, 338 °C, is higher than the melting point of PTFE, 327 °C. PTFE starts getting soft and will be deformed at 260 °C.

Closed-digestion systems have the following additional advantages as compared to the open systems:

- Contamination from surroundings is minimized.
- Loss of volatile species is prevented.
- At elevated temperatures, HNO_3 becomes a better oxidant and thus can be used without the need of any other reagent in many cases. The resulting matrix is preferred for most of the techniques such as ETAAS, ICP-AES and ICP-MS.
- Since relatively lower amounts of reagents are used, contamination is minimized and resulting matrix is simpler.

Digestion bombs have one important disadvantage; the amount of sample is usually limited to 0.5 g. Therefore, depending on the final volume of dissolved sample, resulting analyte concentration may be too low with respect to the limit of quantitation of the analytical technique to be applied. In such a case, the need for a more sensitive technique will directly elevate the cost of analysis.

The digestion bombs are designed to have the weakest part at the bottom, so that any accidental, undesired explosion will cause minimum damage. Some bombs have relief mechanisms to open if internal pressure exceeds a limit.

In the last 10 years, microwave heating has replaced conventional heating in most laboratories for sample digestion. In case of microwave heating, the jacket around the PTFE vessel should be transparent to the radiation; PTFE or other fluorinated polymers such as PFA, perfluoroalkoxy fluorocarbon are used. The bomb should not contain any metal parts since metals absorb and are thus not transparent to microwave radiation. Some manufacturers provide sample vessels made of TFM, a chemically modified form of PTFE, with a smoother and harder surface and lower permeability to gases as compared to ordinary PTFE.

Some compounds including water absorb microwave radiation. Radiation energy is used by dipole rotation and ionic conductance; this causes rapid heating of the absorbing bodies. Since the jacket and vessel are transparent to microwave radiation, temperature rises much faster as compared to conventional oven where transfer from oven to jacket, vessel and sample takes some time. Therefore, the digestion requires a shorter period of time in microwave ovens.

In some microwave-heated bombs, there is a safety membrane that is ruptured if excessive pressures are formed. The membrane is placed in the upper part of the bomb, so that sample loss is minimized. In some other systems, there are temperature and pressure sensors to control the power applied. One manufacturer produces bombs that will open briefly to release some gas in case of pressure build-up; a special ring and spring system then causes very rapid closing after release. However, the indicator ring changes its original position so that the user can be informed if there had been a gas release during digestion procedure (Figure 4.1). Although the time allowed for gas release is very short, the user then decides whether the contents should be used for subsequent analysis or not. If the decision is negative, the digestion may be repeated using a fresh sample and a programme of lower temperature.

Microwave ovens manufactured for digestions in a chemistry laboratory are more expensive, but also more sophisticated than the ones used in kitchen. Regarding this comparison, the following useful features should be mentioned:

- There are gas sensors with a feedback electronic system to cut off power in case of leakage from the bomb.
- Mechanical strength of walls to protect laboratory personnel in case of an explosion.
- Temperature and pressure sensors to monitor inside of the bombs to provide safety and control over the digestion procedures.
- Fume exhaust system.
- Rotation of vessels to homogenize the exposure to microwave radiation.

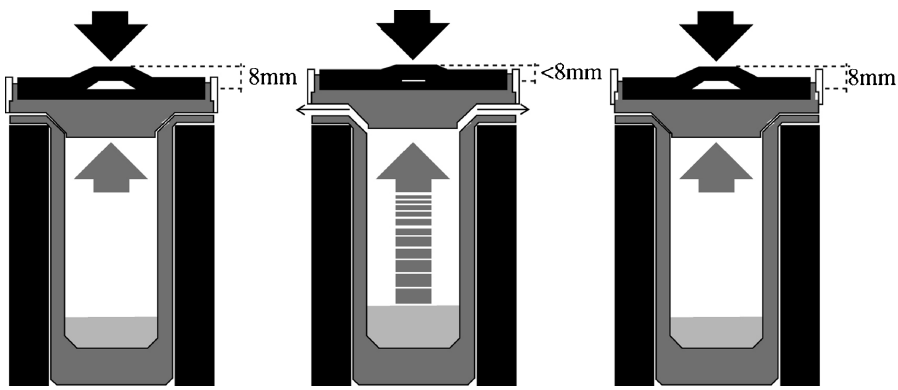


Figure 4.1 *Operating principle of gas release and informing the user in microwave heated digestion bombs (Adapted from Ref. 10 with permission from Mr. Francesco Visinoni and Milestone S.r.l., Italy[10])*

Because of the advantages mentioned above, close digestion by microwave ovens has become the method of choice in many laboratories.

As a concluding remark for this section, one should be reminded that sample decomposition by acid attack is mostly based on trial and error; previous experience including relevant literature must be always considered.

Some digestion procedures using microwave heating for several food samples are given below:

Rice flour[11]

- Weigh around 1.0 g of powdered sample, place in TFM vessel.
- Add 5.0 mL of conc. HNO_3, 1.0 mL of conc. H_2O_2 and 5.0 mL of H_2O; gently swirl the mixture to homogenize.
- Close the vessel, using 1000 W power; apply the following heating regime:

Time (min)	*Temperature (°C)*
3	25–85 (ramp)
9	85–145 (ramp)
4	145–180 (ramp)
15	180

- Cool to room temperature before opening the vessels.
- The cooled solution is ready for further handling for analysis.

Wheat flour[11]

- Weigh around 0.5 g of powdered sample, place in TFM vessel.
- Add 6.0 mL of conc. HNO_3 and 2.0 mL of conc. H_2O_2; gently swirl the mixture to homogenize.
- Close the vessel, using up to 1000 W power; apply the following heating regime:

Time (min)	*Temperature (°C)*
6	25–200 (ramp)
15	200

- Cool to room temperature before opening the vessels.
- The cooled solution is ready for further handling for analysis.

Milk powder[11]

- Weigh around 1.5 g of sample, place in TFM vessel.
- Add 12.0 mL of conc. HNO_3; gently swirl the mixture to homogenize.
- Close the vessel, using up to1000 W power; apply the following heating regime:

Time (min)	*Temperature (°C)*
10	25–180 (ramp)
10	180

- Cool to room temperature before opening the vessels.
- The cooled solution is ready for further handling for analysis.

Mushrooms[11]

- Weigh around 0.5 g of sample, place in TFM vessel.
- Add 8.0 mL of conc. HNO_3 and 2.0 mL of H_2O_2; gently swirl the mixture to homogenize.
- Close the vessel, using 1000 W power; apply the following heating regime:

Time (min)	*Temperature (°C)*
7	25–100 (ramp)
7	100–200 (ramp)
7	200 (ramp)

- Cool to room temperature before opening the vessels.
- The cooled solution is ready for further handling for analysis.

References

1. N.T. Crosby and I. Patel, *General Principles of Good Sampling Practice*, Royal Society of Chemistry, London, 1995.
2. FAO/WHO-EFP/83.53, *Guidelines for the Study of Dietary intakes of Chemical Contaminants*, World Health Organization, Geneva, 1983.
3. National Academy of Sciences, *Recommended Dietary Allowances*, 10th edn, Washington, DC, 1989.
4. OECD, *Food Consumption Statistics* 1964–1978, Paris, 1981.
5. T.S.M. Van Schaik and L.M. Dalderup, A study in food consumption in eleven areas of the European Communities in connection with radioactive contamination, notably in Friesland, *Voeding*, 1968, **28**, 1–15.
6. P. Koivistoinen (ed), Mineral element composition of Finnish foods. *Acta Agriculturae Scandinavica Suppl*., 1980, **22**, 1–171.
7. T. Mumcu, I. Gokmen, A. Gokmen, R. Parr and N.K. Aras, Determination of minor and trace elements in Turkish diet by duplicate portion technique, *J. Radioanal. Nuc. Chem*., 1988, **24**, 289.
8. M. Sinisalo, J. Kumpulainen, M. Paakki and R. Tahvonen, Content of major and minor mineral elements in weekly diets of eleven Finnish hospital, *J. Hum. Nutr. Diet.*, 1988, **2**, 43.
9. Analytical Methods Committee, Notes on perchloric acid and its handling in analytical work, *Analyst*, 1959, **84**, 214.
10. ETHOS PLUS User Manual, Revision 1/2000, Milestone Microwave Laboratory Systems, Italy, 2000.
11. ETHOS PLUS Application Notes, Milestone Microwave Laboratory Systems, Italy, 2000.

Further Reading

1. J. Dolezal, P. Povondra and Z. Sulcek, in *Decomposition Techniques in Inorganic Analysis*, D.O. Hughes, P.A. Floyd and M.S. Barratt (eds, English translation), London Iliffe Books Ltd., London, 1968.
2. R. Anderson, "Sample Pretreatment and Separation", *Analytical Chemistry by Open Learning*, Wiley, Chichester, 1991.
3. R. Bock, in *A Handbook of Decomposition Methods in Analytical Chemistry*, I.L. Marr (Translated and Revised), International Textbook Company, Glasgow, 1979.
4. E. Prichard, G.M. MacKay and J. Points (eds), *Trace Analysis: A Structured Approach to Obtaining Reliable Results*, The Royal Society of Chemistry, Cambridge, 1996.
5. D.A. Skoog, D.M. West and F.J. Holler, *Fundamentals of Analytical Chemistry*, Chapters 32 and 33, Saunders College Publishing, New York, 1996.
6. M. Zief and J.W. Mitchell, *Contamination Control in Trace Element Analysis*, Chemical Analysis series, Vol 47, Wiley, New York, 1976.
7. H.M. Kingston and L.B. Jassie, *Introduction to Microwave Sample Preparation: Theory and Practice*, American Chemical Society, Washington, DC, 1988.
8. A.G. Howard and P.J. Statham, *Inorganic Trace Analysis: Philosophy and Practice*, Wiley, New York, 1993.

CHAPTER 5

Spectrochemistry for Trace Analysis

5.1 Fundamentals, Definitions and Terms

Interaction of light with matter in several ways has allowed chemists to develop a group of analytical approaches that may be termed as *spectroscopic* or *spectrometric* techniques. The term *spectroscopy* should be used for rather fundamental studies; for the chemical analysis it is more accurate and proper to use the term *spectrometry*. In common practice, the term *spectrochemistry* includes all the spectrometric techniques for qualitative and quantitative analysis.

Light is an electromagnetic radiation; its dual character includes particle and wave behaviours. The electrical and magnetic components of a light wave propagate on planes perpendicular to each other; only the electrical component is considered to be responsible in interaction of light with matter regarding the spectrochemical behaviour.

Wave behaviour of a monochromatic, plane-polarized (propagating in one plane only) light is shown in Figure 5.1. *The energy E* of light is proportional to its *frequency* ν, which is the number of waves passing through a point per second. The *wavenumber* $\overline{\nu}$ (in cm^{-1}) is the reciprocal of the wavelength, λ. Energy and frequency are related as follows:

$$E = h\nu = hc/\lambda = hc\overline{\nu} \tag{5.1}$$

where h ($= 6.62608\times10^{-34}$ Js) is the Planck's constant and c ($= 2.99792\times10^{8}$ m s^{-1}) is the speed of light in vacuum.

The velocity of light is not a constant and is reduced in a medium i depending on its refractive index, n_i ($= c/\nu_i$) from its value in vacuum, c, to ν_i. In the medium i, $\lambda_i = \lambda_0/n_i$. Therefore, a light wave has various values of ν and λ through different media, and the reduction in wavelength is accompanied by the reduction in speed; but its frequency is always a constant.

$$\nu = c/\lambda_0 = \nu_i/\lambda_i \tag{5.2}$$

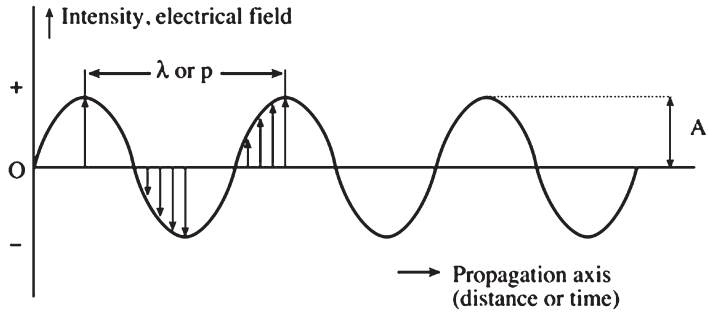


Figure 5.1 *Representation of a monochromatic, plane polarized light radiation. Only the electrical field is considered. λ, wavelength (nm); p, period and A, amplitude*

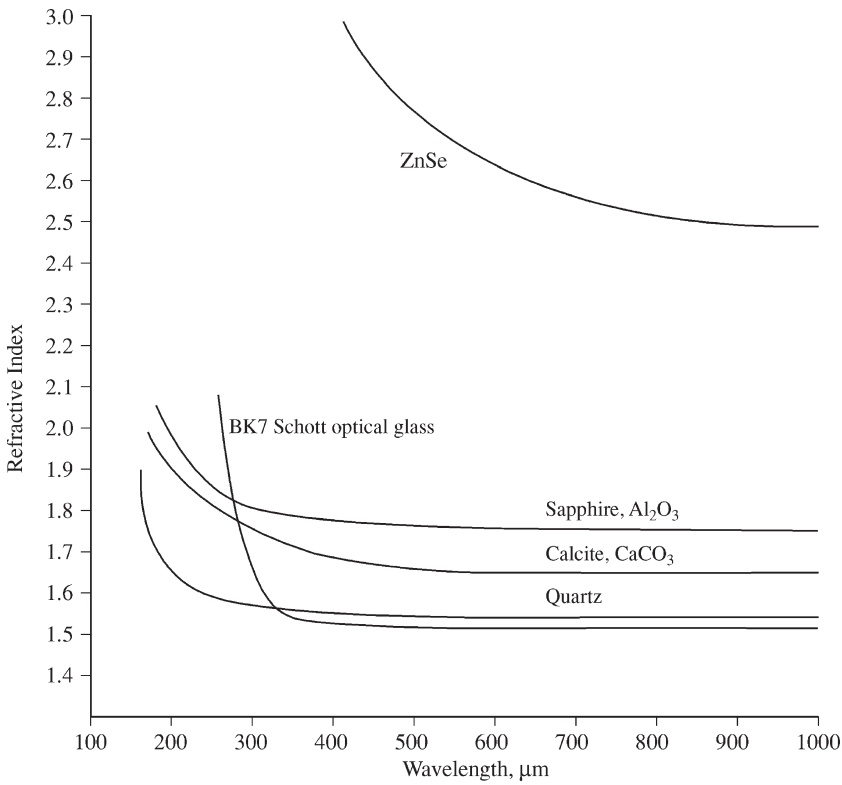


Figure 5.2 *Dispersion curves for some optical materials*

where c and λ_0 are the values for vacuum, and ν_i and λ_i the values for the medium i. Refractive index is also a function of wavelength; a plot of n_i *versus* λ is called a *dispersion curve*; in common spectral regions where the material is transparent, refractive index increases as the wavelength decreases. Dispersion curves for some common optical materials are shown in Figure 5.2.

Regarding the particle character, the term *photon* is used. The power of radiation, P, is proportional to the number of photons reaching an area per second. The sign $h\nu$ is commonly used to denote a photon with a frequency of ν. Electromagnetic spectrum covers a large range of frequencies, starting from cosmic rays at the high-energy end to the radio waves at the low-energy extreme. Spectrochemistry for analysis involve ultraviolet (UV), visible (VIS) and infrared (IR) regions. From spectrometric point of view, UV and VIS are the same, since both of them involve electronic transitions, except that the latter corresponds to the range of human eye perception. These three regions of radiation are commonly combined under the name of *optical spectrometry* (*or spectroscopy*); the behaviour of light in its interactions with matter and therefore spectrochemical instrumentation has great similarities. Regarding trace element analysis, only the UV and VIS radiations are important and these two regions are to be emphasized in this chapter.

In order to have a better understanding of spectrochemical techniques, it is important to consider the common ways in which the light interacts with matter. Light propagates on a line. Normally a light source will emit photons of different wavelengths in a range, with the electronic components propagating on different planes. A group of photons having ideally the same frequency is termed as *monochromatic light*; actually their frequencies in practice can never be exactly the same, but rather form a group with values gathering around a maximum, which is the *nominal frequency or wavelength*. There are instrumental as well as theoretical limitations for obtaining a truly monochromatic radiation. However, with better instrumentation it is possible to obtain a more monochromatic light beam, meaning that the statistical distribution for the frequencies around the nominal frequency has a narrower range; such a beam is said to have a better *spectral purity*.

A *spectrum* is a plot of light intensity versus its frequency. Spectrochemical analysis is based on a sensitive and selective response of a sample spectrum. Selectivity is based on the fact that atoms and molecules have their specific energy diagrams for electronic, vibrational and rotational levels; therefore, each species will interact with light at some specific wavelengths: The *qualitative analysis* is based on the *position* of signals on wavelength axis. The quantitative *analysis*, on the other hand, is related to intensity axis; which is a degree of the number of photons involved in spectrochemical interaction, which is in turn a measure of the analyte concentration or quantity. Spectrochemical analyses are based on comparative methods; calibration plots must be used.

When light traverses through a medium, this phenomenon is called *transmission*; a sample that allows the transmission of light at a certain wavelength is called *transparent*. If the light beam is not even partly transmitted, the sample is said to be *opaque.* During transmission, some of the photons may interact with chemical species on their path; if the energy of photons matches the difference between two energy states of these species, the energy of photon may be used to excite the particle under interaction. This process is called *absorption*. Alternatively, some light will be reflected at the interface of air and sample surface. This reflection is at a minimum value for incident rays perpendicular to the interface, and is a function of the angle of incidence with the surface normal and the refractive indices of both media

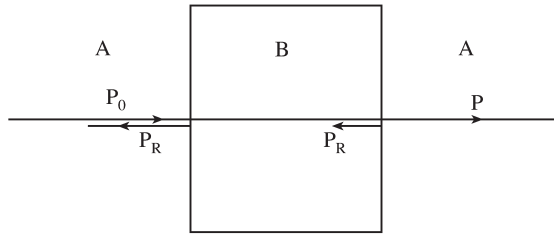


Figure 5.3 *Behaviour of transmitted monochromatic light through a partly absorbing sample where the beam is perpendicular to all the interfaces. A, air ; B, transparent medium; P_0, incident ray power; P, transmitted ray power and P_R, reflected beam power*

at the sides of the interface. The following terms are used regarding transmission, absorption and reflection (Figure 5.3).

$$\text{Transmittance } T = P/P_0 \tag{5.3}$$

$$\text{Percent transmittance } \%T = 100(P/P_0) \tag{5.4}$$

$$\text{Reflectance } R = P_R/P_0 \tag{5.5}$$

$$\text{Absorbance } A = -\log T = \log(P_0/P) \tag{5.6}$$

A rarely used term, *absorptance*, is a measure of radiant energy absorbed, $\alpha = [(P_0 - P - P_R)/P_0]$. Ideally, in any case where monochromatic light is partially transmitted, $R + \alpha + T = 1$. When the incident beam is not perpendicular to the interface, refraction will take place together with the transmission; the path of light will be altered according to the *Snell's law*,

$$n_1 \sin \theta_1 = n_2 \sin \theta_2 \tag{5.7}$$

where n_1 and n_2 are the refractive indices of media (1) and (2), and θ_1 and θ_2 the angle between the surface normal and the incident and the refracted beams, respectively. Therefore, a medium with a refractive index larger than that of air will refract the oncoming beam in such a way that it will become closer to the surface normal. If, on the other hand, the beam is approaching from the denser medium, refraction occurs until a critical angle, θ_c, is reached for the value of θ_2; at this point, θ_1 equals to 90°; at this point the beam is refracted along the interface. For the values of θ_2 exceeding θ_c, *total internal reflection* takes place and the incident beam is reflected back into the same medium with an angle of reflection $\theta_2' = \theta_2$, as shown in Figure 5.4.

In order to correct for reflection at interfaces and scattering, both at the sample container and the sample itself, absorbance *A* is always measured against a proper blank, as shown in Figure 5.5. Depending on instrumentation, as will be discussed, this measurement may be performed in separate cases or simultaneously.

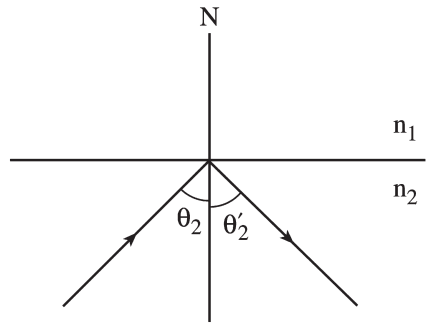


Figure 5.4 *Total internal reflection. N, normal to interface; n_1, refractive index of the rare medium n_2, refractive index of the dense medium and $n_2 > n_1$; $\theta_2' = \theta_2$*

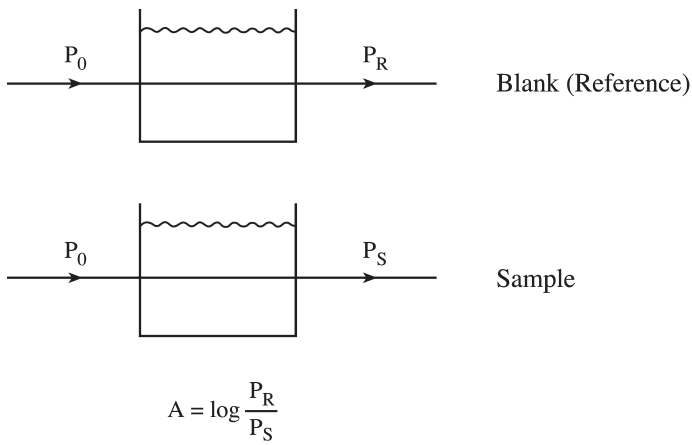


Figure 5.5 *Absorbance measurement of a sample solution against a blank*

Another important kind of light-matter interaction is *scattering*. If a photon reaches a particle with dimensions significantly smaller than the wavelength of light, the photon is absorbed and reemitted mostly on the same axis on which the incident ray propagates. This process is called *secondary emission*; its half-life is about 10^{-15} s. In this case, practically no net change occurs for the photon and it continues its propagation on its original axis. However, a very small part of energy is propagated at all angles and the energy of photon continuing on its unaltered path slightly decreases; this is called *scattering*. As the dimensions of the particles increase and becomes comparable to the wavelength, scattering becomes more important and reaches to a point where the particle becomes a new light source propagating the oncoming photon's energy in all possible directions. On a reflecting surface, similar phenomenon takes place; if the imperfections on a smooth surface has dimensions much lower than the oncoming photon's wavelength, almost total reflection takes place; angles of the incident and the reflected beams with the normal are equal; this

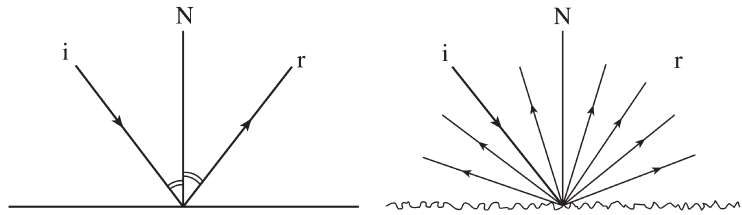


Figure 5.6 *Diffuse (a) and specular (b) reflections. N, normal to the surface; i, incident ray and r, reflected ray*

is called *specular reflection*. If, however, the imperfections on surface are comparable to the wavelength or larger than it, the beam is reflected in all possible directions; this is called *diffuse reflection*. Therefore, a mirror and a white paper both reflect all the visible wavelengths oncoming from a white light source; however, with the former, an image is formed since specular reflection takes place, where no image is formed for the latter because of diffuse reflection. These two kinds of reflections are shown in Figure 5.6.

In practice, scattering is usually an important source of error in spectrochemical measurements; high-quality optics and an analysis environment free of dust particles will reduce this effect, proper blanks are often required to minimize the scattering errors.

Regarding the properties of electromagnetic radiation, another important phenomenon is *light polarization*. A normal light source will emit photons having frequencies in a range; these photons will correspond to light waves propagating on different planes as viewed by the receiver. Light waves propagating on a single plane are said to be *plane polarized*. Plane-polarized radiation may be obtained by polarizers; these are optically transparent substances absorbing photons propagating only in one plane, allowing the radiation in the plane perpendicular to the plane of absorption to be transmitted. Phenomenon of plane polarization of light is shown in Figure 5.7; the beam in "c" is transmitted through a polarizer that selectively absorbs the components on *YZ* plane only to obtain plane-polarized light.

Absorbance of a photon will cause the formation of excited state of the analyte species,

$$M + h\nu \rightarrow M^* \tag{5.8}$$

where M and M* are the ground state and excited state of analyte species, respectively; in UV and VIS regions these correspond to electronic states. The excited electronic state, M*, will have half-life of $10^{-8}-10^{-9}$ s. After the absorption of a photon, one of the following takes place:

(i) Radiationless relaxation

$$M^* \rightarrow M + \text{heat} \tag{5.9}$$

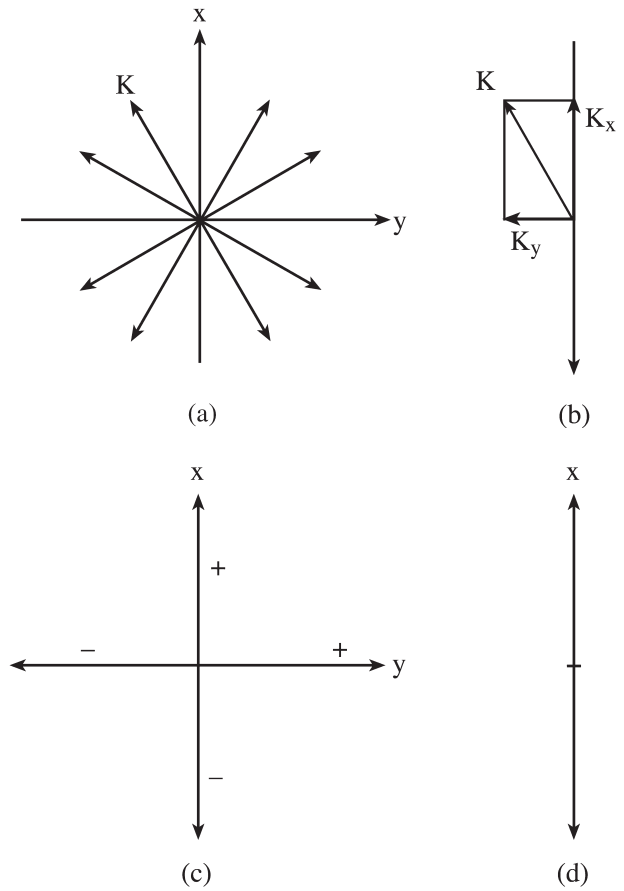


Figure 5.7 *Polarization of light: (a) Several electrical vectors of radiation propagating on Z-axis perpendicular to page. (b) The resolution of the vector K in plane XY into the components of K_x and K_y. (c) The schematic resultant of all the vectors resolved as in (b), unpolarized light. (d) Light propagating in XZ plane only, plane-polarized light*

(ii) Photochemical decomposition

$$M^* \rightarrow A + B \tag{5.10}$$

(iii) Emission of radiation

$$M^* \rightarrow M + h\nu \tag{5.11}$$

or

$$M^* \rightarrow M + h\nu' \tag{5.12}$$

The last process above is called *photoluminescence* when excitation was realized by absorption of radiation. Photoluminescence is called *fluorescence* when the emission

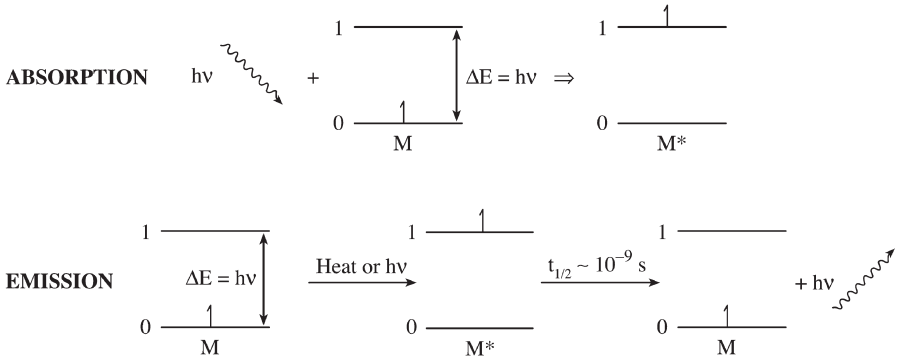


Figure 5.8 *Simplified energy transition processes for absorption and emission of radiation. (0) and (1) are the electronic energy levels. M and M* are the ground and excited states, respectively*

is due to a transition from a singlet excited state (S_1) to a singlet ground state (S_0); the half-lives are 10^{-4} or shorter. On the other hand, if the species go from a singlet excited state (S_1) to a triplet excited state (T_1) prior to emission of a photon and relaxing to a singlet ground state (S_0), this is called *phosphorescence*; the life times associated are 10^{-4} s or longer. All of these phenomena, absorption, emission and luminescence will be discussed in more detail in the following sections. The principles of absorption and emission are schematically shown in Figure 5.8. It must be noted that both emission and luminescence involve emitting of a photon from analyte species. However, depending on the mode of excitation and the energy states involved, different terms are used. The term emission is reserved for the cases where the excitation is collisional in a usually high-temperature environment such as a flame, arc or plasma; the term *thermal emission* is also used. Since at such high temperatures all molecular bonds will be virtually broken, analyte species are only atoms or atomic ions. On the other hand, luminescence methods mostly involve molecules at room temperatures or at cryogenic conditions. Nevertheless, atomic fluorescence is also possible and is used as another spectrochemical technique for trace analysis.

Emission signal from an atomic cloud is dependent on the number of excited atoms. Population of excited-state species is related to temperature and the energy difference between two energy levels involved. This relation is given by Boltzmann equation:

$$N_1/N_0 = (g_1/g_0)\, e^{-\Delta E/kT} \tag{5.13}$$

where N_1 and N_0 are the number of species at excited (1) and ground (0) states, respectively; g_1 and g_0 are the associated statistical factors; ΔE the energy difference between two levels; T the temperature in Kelvin and k the Boltzmann constant that has a value of 1.38066×10^{-23} J K^{-1}. From Equation (5.13), it can be observed that the population of excited states increases with higher temperatures and decreases with increasing energy gap. Since emission signal is directly related to

the number of excited atoms, sensitivity increases with higher temperatures and decreases with higher energy difference between two levels or shorter wavelengths associated.

Blackbody radiation is another important characteristic of some substances related to their optical behaviour. Some solids with high melting and boiling points can be heated up to relatively high temperatures: at these elevated temperatures, these substances will glow in a continuous manner regarding light frequencies. The spectra of this continuous radiation exhibit features characteristic of temperature of the heated substance rather than its chemical properties. This radiation is called *blackbody radiation*. It has the following characteristics:

(i) The wavelength maximum of this radiation is inversely proportional to the absolute temperature.
(ii) The total energy emitted per unit area and time by the blackbody is proportional to the fourth power of temperature.
(iii) The power of emission at a given temperature is inversely proportional to the fifth power of wavelength; this behaviour is valid for the wavelengths larger than the maximum wavelength of the continuous spectrum.

Blackbody radiation is often used to obtain light sources for spectrochemical measurements. It is also important in atomic emission measurements when the hot atomization/excitation medium may emit a continuum to give a background on which the analytical lines are positioned. Some blackbody radiators and their emission features are shown in Figure 5.9.

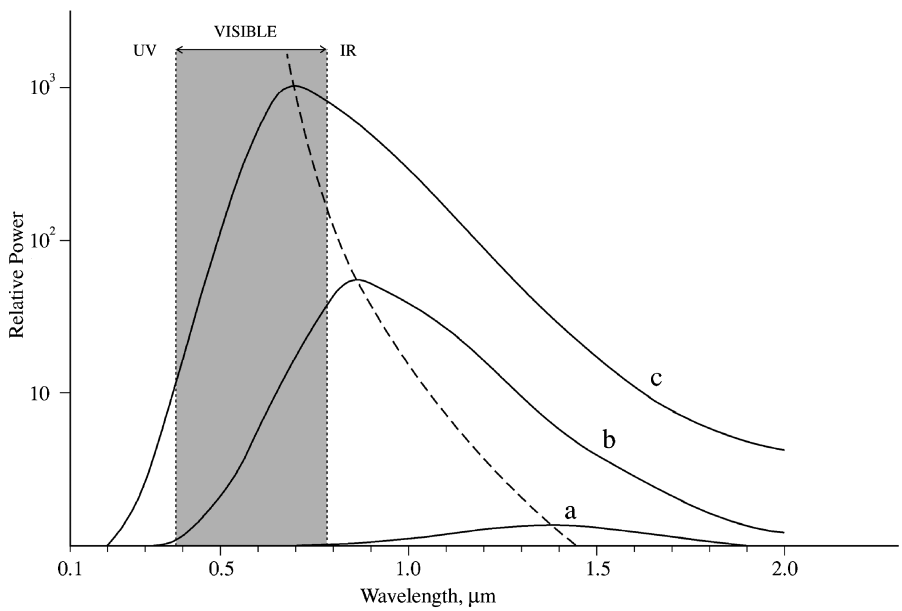


Figure 5.9 *Some examples of blackbody radiation; broken line shows wavelengths at maximum power versus temperature; a, Nernst glower, an infrared source at 2000 K; b, W-lamp at 3300K; c, carbon arc at 4100 K*

5.2 Atomic and Molecular Spectrometry

Molecular electronic energy levels include vibrational and rotational states since molecular analytes have chemical bonds whose vibrations and rotations refer to quantized energy levels. Therefore, molecules will absorb and emit in UV, VIS and IR regions where the transitions for atomic analytes are limited to UV and VIS only. As a result, atomic transitions will be characterized by well-defined energies resulting in narrow peaks, called as *lines*; atomic spectra will consist of lines. Molecular electronic transitions will also involve vibrational and rotational energies in IR region; one of the most powerful spectrochemical techniques for molecular determination is thus *infrared spectrometry*. Electronic spectra for molecules will involve transitions between the several vibrational and rotational levels of both ground and excited energy states; therefore, the energy difference between these states is not as well defined as in the case of atomic states; the peaks will be broader and they are called *bands*; a molecular spectrum will consist of bands. In gaseous state these molecular signals are resolved relatively better, but in liquid and solid states the peaks will have a lesser structure and individual lines may broaden sufficiently to form spectral bands. Half-widths of molecular absorption bands are typically 50–200 nm, while for atomic lines this value is well below 1 nm.

Absorption, emission and luminescence are three basic modes of spectrochemical measurements. While the modules of instruments for wavelength selection and measurement of light intensity are very similar to each other, the relative positions of the sample and light source have differences as schematically shown in Figure 5.10.

Absorption mode in spectrochemistry involves the measurements of the degree of light absorption by analyte species in sample at a monochromatic wavelength. In the emission mode, the analyte species are excited by thermal means such as flame, arc or plasma and the photons emitted are detected. In the case of luminescence, the analyte species are excited by a light source usually in a direction perpendicular to the axis

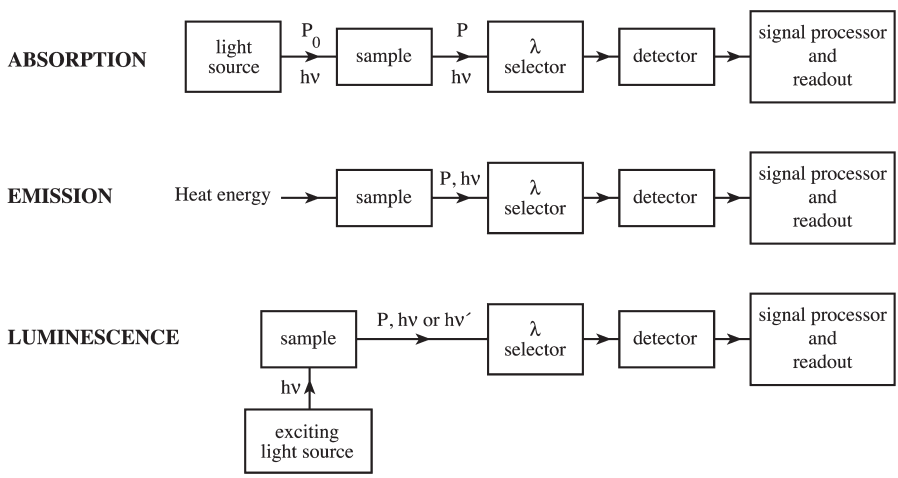


Figure 5.10 *Schematic representation for absorption, emission and luminescence modes in spectrochemistry*

along which the emitted photons are detected; the axes for light detection and excitation need to be different, so that exciting beam photons do not reach the detector.

It must be noted that while emission and luminescence measurements are very similar, the absorption mode is quite different. For the cases of emission and luminescence, higher number of photons reaching the detector would indicate larger number of analyte species under interaction. While for the absorption mode, smaller number of photons at detector would indicate a large portion of radiation was absorbed, meaning that a high quantity of analyte is present.

5.2.1 Molecular Spectrometry

In absorption measurements, analyte concentration and absorbance are related by the *Beer's law*,

$$A = \varepsilon bc \tag{5.14}$$

where A is the absorbance, ε the molar absorptivity (cm^{-1} mol^{-1} L), b the light path in the sample (cm) and c the analyte concentration (mol L^{-1}).

The above relation is valid for monochromatic light. Ideally, a plot of A *vs*. c will be a line with a slope of εb. The linearity may not be obeyed at concentrations typically above 0.01 mol L^{-1}; other reasons for the deviation from linearity are chemical equilibria and instrumental imperfections to provide truly monochromatic radiation for the measurements. Light path, b, is a constant and typically 1.0 cm for UV and VIS molecular absorption measurements for solution samples. Slope of calibration plot or *sensitivity*, therefore, depends on ε, a value specific to the analyte species. Molar absorptivity, ε, is a measure of probability of absorbing a photon whose energy matches the energy difference between the ground and excited energy levels. Beer's law is valid for UV, VIS and IR regions.

Spectrochemistry for element determination is related to electronic transitions and thus concerns only the UV and VIS regions. Molecules can be qualitatively and quantitatively determined by absorption measurements. In order to determine elements in aqueous solutions, the following steps should be carried out:

1. The analyte element may be present in different chemical forms, oxidation states, complexes, *etc*. These forms are converted into a single form such as a cation in single valency.
2. A reagent is introduced to react with analyte to form a light-absorbing product.
3. The reaction between the reagent and analyte should be highly selective and should have a large equilibrium constant. Therefore, if a sufficiently excess amount of reagent is used, final concentration of the product is directly proportional to the initial analyte concentration.
4. Absorbance of the product is measured using a Beer's law calibration plot and the analyte concentration in sample is determined.

A typical example is the determination of iron in aqueous solutions by complexing Fe(II) ions with 1-10-phenanthroline to form a complex whose absorbance is measured at 515 nm.

There is a large group of analytical methods for element determination based on the principles above; these are commonly called *spectrophotometric methods*, although this term is not theoretically limited to this type of analysis. After the development of atomic spectrometric techniques, spectrophotometric methods for element determination has become less popular; however, for most non-metals this approach is still a powerful technique.

5.2.2 Luminescence Spectrometry

Excitation of atoms or molecules by an external light source, followed by the measurement of fluorescence or phosphorescence spectra constitutes the basis of *photoluminescence* techniques. Excited species to emit light may be alternatively produced by chemical or biological reactions on which the *chemiluminescence* and *bioluminescence* spectrochemical techniques are based. Luminescence techniques have not gained popularity in general as much as the absorption and emission approaches. Regarding molecular analysis, there are many methods developed based on luminescence. Numerous biochemical and clinical methods employ the high selectivity and sensitivity of luminescent species. These methods may be used for determination of elements in ways similar to those in spectrophotometric methods. For example, quantitation of Se may be based on the fluorescence measurement of its complex with a selective reagent, diaminonaphtalene (DAN).

5.2.3 Atomic Spectrometry

Group of techniques to determine elements in their atomic state may be named as *atomic spectrometry*. Analyte species usually in solution or in solid form are introduced into a thermally and/or electrically energetic medium such as a flame, arc or plasma, where molecular bonds are broken. Once the analyte species are converted into free atoms and atomic ions, their determination may be carried out by atomic absorption, atomic emission or atomic fluorescence techniques, of which the first two are more commonly employed today. The fundamental difference between the atomic emission and atomic fluorescence is that in atomic emission, the analyte excitation to higher electronic state is collisional and in atomic fluorescence, it is realized by excitation from an external radiation source. In atomic absorption, the relation for quantitation is similar to Beer's law.

$$A = kC \tag{5.15}$$

where, k is a proportionality constant, which includes not only the probability of absorption, but also the efficiency of means to introduce analyte atoms into the light path. Similar relations for atomic emission and atomic fluorescence are used for calibration plots. In the following sections atomic spectrometric methods will be handled in more detail.

5.3 Instrumentation

Optical spectrometry, which covers the spectral regions of UV, VIS and IR, employs very similar means of instrumentation for absorption, emission and luminescence

techniques. However, there are important differences regarding the *window* materials through which the radiation should be transmitted. In addition, photoelectric detectors dominate the field for UV and VIS, while heat detectors are commonly used in IR region. In this section, instrumentation regarding UV and VIS regions will be emphasized.

5.3.1 Basic Components for Spectrometric Instrumentation

5.3.1.1 Some Important Optical Units

In an optical instrument there is often a need for *window materials*. These are employed as cells to contain liquid samples for molecular absorption or luminescence measurements or for protection of optical units from dust and dirt. A *window material* should be highly transparent in the region of interest. Glass is transparent between the wavelengths of 380 and 2000 nm and is commonly used in VIS region. Fused silica and quartz are transparent between 180 and 3500 nm and are the choice of window material in the UV region. Chemical and physical inertness is also important for a window material, especially when used as a sample cell.

Light propagates on a line. Spectrometric instruments require direction, focusing and defocusing of a light beam for its proper use, such as passing it through the sample and sending it to a detector in optimum geometry. It is essential to be able to follow a light path within an optical instrument and comprehend how the system is working. This knowledge is necessary to understand the subtle differences in instrument designs and will allow the user to have a better control of their capabilities, functions and errors.

Mirrors are used to reflect and direct a light beam. In contrast to the ones used for domestic purposes, mirrors used in analytical instrumentation are front-surface coated, using a reflective metal, such as Al or Au. A very thin layer of protective quartz coating has become popular in the last decade and is very functional to prevent mechanical damages to surface as well as its oxidation. A *half mirror* is partially coated so that about 50% transmittance and 50% reflectance in a spectral range are obtained. Sometimes the coating is performed in the form of small islands of circles or squares on the substrate surface; therefore the total area of the reflecting surfaces amounts to about 50% of the total; these devices are called *grid-type mirrors* and function as half mirrors. Both of the devices described above may be employed to split a beam or to recombine beams into a single one; the terms *beam splitter* or *beam recombiner* may be used for these, respectively; these are shown in Figure 5.11.

Half mirror can separate a beam into two new beams only in *space*, not in *time*. A *chopper* or *sector mirror* can separate a beam into two beams both in space and in time (Figure 5.12). A chopper, therefore, produces two light beams in an intermittent character; they are said to be modulated. *Modulation of light signal* after detection produces a modulated electrical signal, which is called alternating current (ac) whose handling is advantageous as compared to a direct current (dc) signal.

Modulation is also often realized by powering a light source intermittently in an ac manner, such a source of radiation is then called *a pulsed light source*. Therefore pulsing, mirrors, half mirrors and choppers may be used in several combinations to obtain different ways to function in spectrometric instruments.

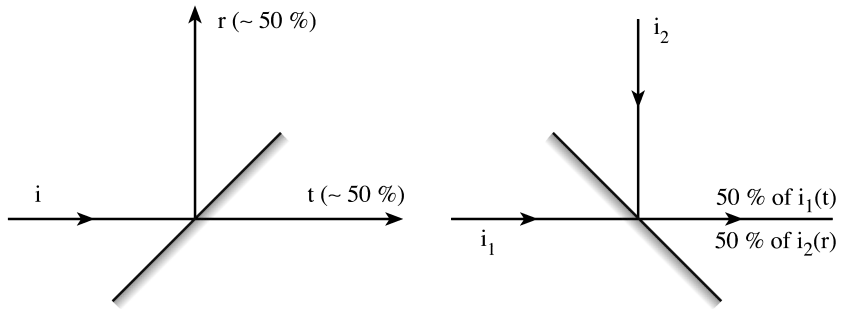


Figure 5.11 *Several uses of half mirrors: (a) half mirror used as a beam splitter. (b) half mirror used as a beam recombiner*

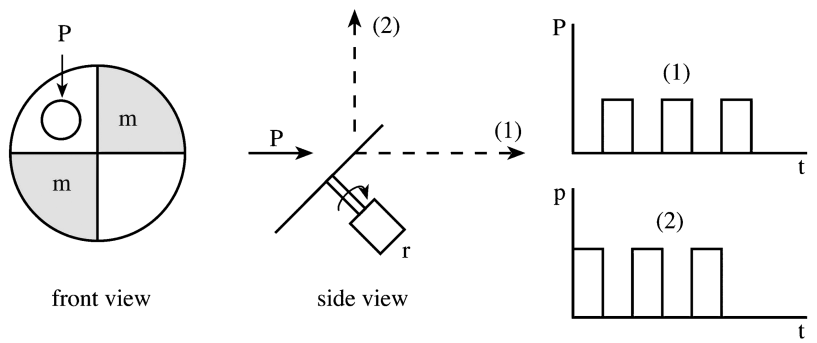


Figure 5.12 *A simple rotating chopper used to separate a light beam both in space and in time. P, position of light beam on sector mirror; m, mirror sections; r, rotating motor; o, oncoming beam and 1,2, separated beams on P (power) vs. t (time) coordinates*

While the half mirrors cannot produce modulation, they are immobile and thus cause no mechanical and/or electrical noise or vibrations in contrast to a mechanical chopper. On the other hand, splitting a beam using a half mirror causes about 50% loss in light power. A chopper should theoretically have no such loss and both the reflected and traversing beams should retain their original powers; however, in practice, the dust accumulation aided by static electricity on rotating mirrors causes significant scattering losses on the amount of light specularly reflected; therefore the beam reflected by a chopper mirror has a reduced power.

All the common optical laws for lenses are valid for *paraxial rays* only; these are the rays parallel and propagating very close to the optical axis. Lenses and mirrors with curved surfaces are employed for focusing purposes. Ideally, a convex lens will focus the paraxial rays at its focal point. However, since refractive index varies with wavelength, different images for different wavelengths are formed along the optical axis around the focal point. This phenomenon is called *chromatic aberration*; its practical correction is possible but rather limited to certain wavelengths at a time. Therefore, concave mirrors are preferred to lenses in general for beam focusing.

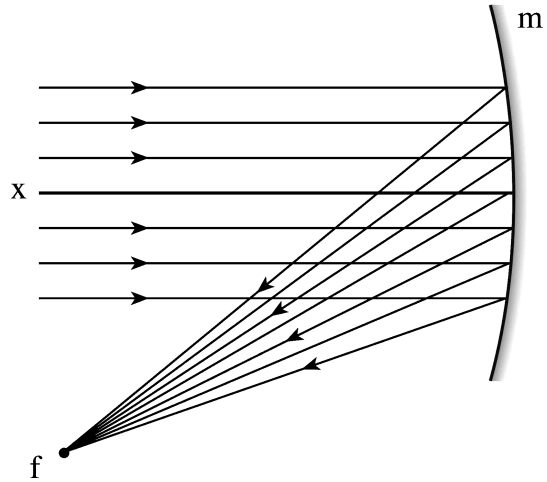


Figure 5.13 *An off-axis concave mirror commonly used in spectrometric instruments. m, collimating off-axis; x, optical axis and f, focal point*

An off-axis concave mirror is commonly used in spectrometer designs; its functioning is shown in Figure 5.13.

The mirror shown in Figure 5.13 functions in two ways: the rays approaching from the focal point to the mirror will be rendered parallel to the optical axis; this is called *collimation*. On the other hand, the collimated rays approaching to the mirror will be focused on the focal point. These functions are often used in monochromators, the most common wavelength selectors.

5.3.1.2 Wavelength Selectors

In order to realize optical measurements at a selected wavelength, detection of radiation confined to a narrow spectral band or line is required; this confined distribution in practice is called *monochromatic*. It should be remembered, however, that true monochromatic light cannot be obtained. There are several ways to obtain monochromatic radiation.

Filters are simple devices. An *absorption filter* contains properly designed chemicals either imbedded in or sandwiched between window plates; light is absorbed at undesired wavelengths, leaving a passage of monochromatic light at the wavelength of interest; this device is called a *bandpass filter*. Alternatively, filters may absorb the radiation selectively below or above a threshold value of wavelength; these devices are called *cut-off filters.* Quartz and glass are two natural cut-off filters. Nominal wavelength, effective bandwidth or full-width at half-maxima (FWHM) and per cent transmittance at the nominal wavelength are three important figures to characterize a bandpass-type filter; these are shown in Figure 5.14.

Interference filters function via optical interference phenomenon; the structure and principle of obtaining a monochromatic band is shown in Figure 5.15. Dielectric layer is typically MgF_2 or CaF_2. The beams being transmitted through an interference filter

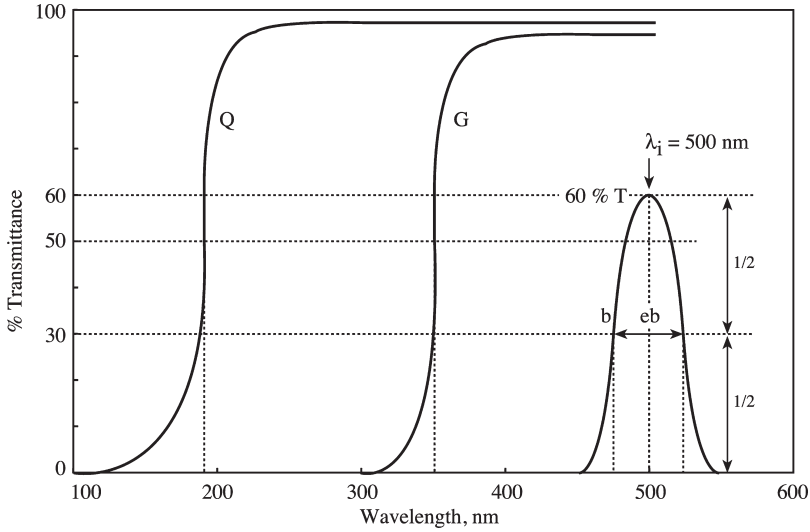


Figure 5.14 *Absorption filters. Q, quartz as a cut-off filter, 50% T at 180 nm; G, glass as a cut-off filter, 50% T at 350 nm and B, bandpass filter, nominal wavelength is 500 nm, 60% T at 500 nm, effective bandwidth, eb, is 40 nm*

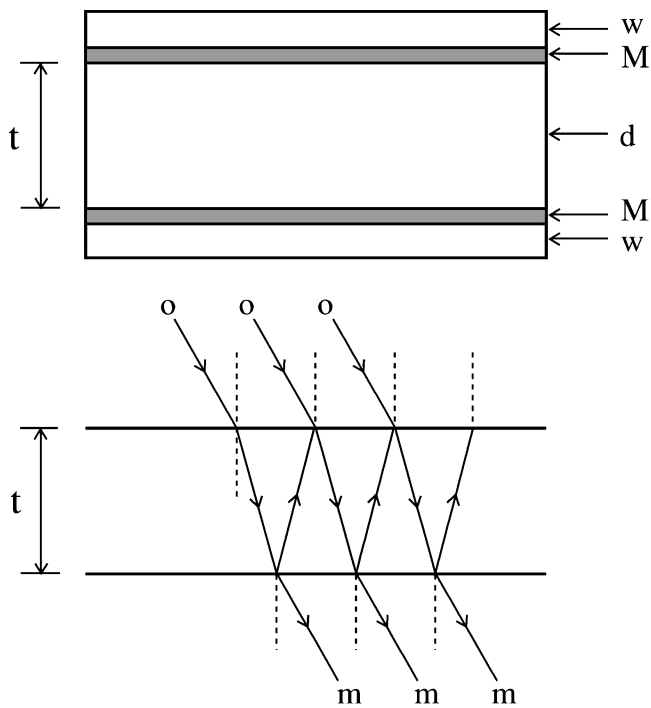


Figure 5.15 *Interference filters. o, oncoming radiation, spectrally continuous and m, monochromatic band of radiation; w, window; M, metal layer; d, dielectric layer, MgF_2 or CaF_2; t, thickness of dielectric layer*

are those which form constructive interference where their path difference is a multiple of a wavelength. It can be shown that

$$\lambda = 2tn/N \tag{5.16}$$

where t is the thickness of the dielectric layer in nm, n its refractive index and N, an integer, is the *order of interference*. Therefore an interference filter whose value of $2tn$ matches 600 nm will transmit the wavelengths of 600, 300 and 200 nm for the values of $N=1$, 2 and 3, respectively. The wavelengths at higher orders, such as 150 and 120 nm, will be stopped by quartz windows. Undesired wavelengths at any order can be eliminated by using a proper cut-off filter. Interference filters are available in UV, VIS and IR regions up to about 14 λm. Absorption filters have effective bandwidths that range from 20 to 300 nm, where interference filter bandwidth values range from 0.2 to 1.5% of the nominal wavelength, amounting to a range of 0.4–12 nm in UV–VIS region. In general, the bands obtained through interference filters are more monochromatic, but the maximum transmittance values are lower as compared to absorption filters. Therefore, in general, interference filters provide better spectral purity associated with higher costs when compared to absorption filters.

Prisms and *gratings* are devices that are capable of dispersing a continuous radiation into its monochromatic components. *Prisms* are transparent materials that are cut with perfectly polished faces; they disperse light, because refractive index is dependent on wavelength of radiation as shown in Figure 5.2. Since the slope of the curves in this figure is not constant, dispersion by a prism is not linear, being better for the lower wavelengths of a spectral region where the change in refractive index per unit change of wavelength is larger. Schematic representation of dispersion by a prism is shown in Figure 5.16.

Gratings are surfaces with fine-ruled parallel grooves on them; they may be of *transmission* or *reflection* type; and the latter is more popular. Reflection gratings cause the oncoming parallel rays to undergo *diffraction*, so that outcoming rays will be of the same wavelength when they form constructive interference. The condition

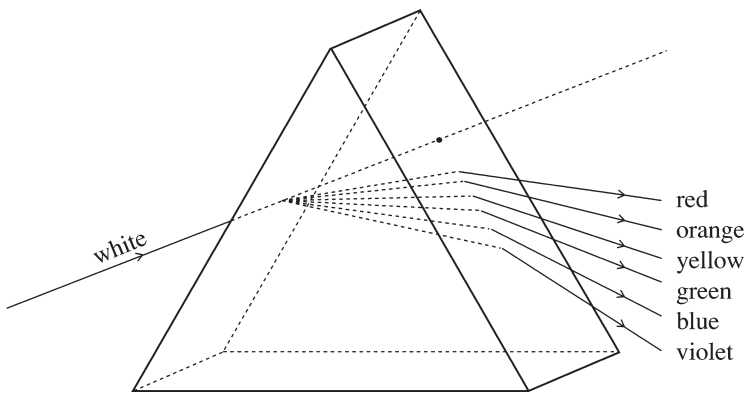


Figure 5.16 *Schematic representation for dispersion of white light into its monochromatic components by a prism*

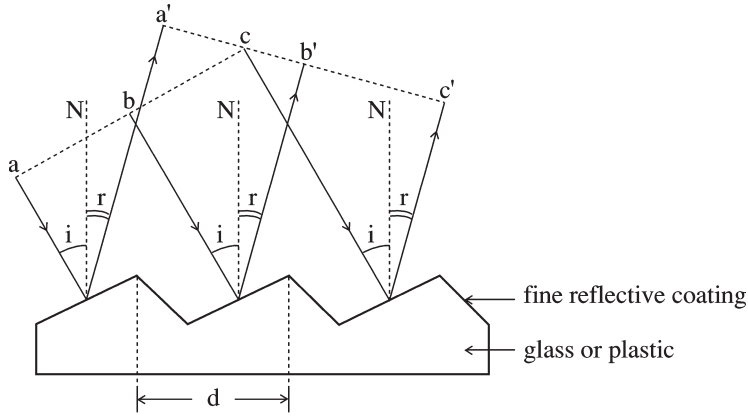


Figure 5.17 *Diffraction on a reflection grating surface. Outcoming rays are parallel to each other have the same wavelength. GN, grating normal; i, angle of incidence and r, angle of reflection*

for constructive interference for a particular wavelength is shown in Figure 5.17 and can be expressed by the grating formula

$$N\lambda = d\,(\sin i \pm \sin r) \tag{5.17}$$

where λ is the wavelength, N, an integer, is the order of diffraction, d the distance between the grooves (lines), i is the angle of incidence and r is the angle of reflection. The angle of reflection has a positive sign when both the incident and the reflected rays are on the same side of the grating normal; otherwise a negative sign is assigned to this term. Dispersion by a grating is almost linear on a scale of wavelengths; this property is in contrast to non-linear dispersion by prisms. While linear dispersion is an advantage for gratings, they have the problem of high orders in a manner similar to the interference filters; there are no order problems for prisms.

Prisms and gratings can disperse the polychromatic light into its monochromatic components, but they are not capable of forming images. While a spectrum is measured, radiation must be dispersed into the monochromatic components with bandwidths as small as required. A *monochromator* is a device that can use a polychromatic (*white*) image and form many monochromatic images to be used for spectral measurements at different wavelengths. One should be reminded that the term *white* may be used instead of continuous radiation, while it means really white only for the VIS region; in other regions it refers to the sum of all the frequencies covered by that region.

A typical monochromator has the following components:

1. A dispersing device, which is a prism or a grating.
2. *Entrance slit*: this is the start of the optical journey in a monochromator. A white image is focused on the entrance slit

3. *Collimators*: these are lenses, or more commonly, mirrors. These units realize focusing and defocusing as required, so that the monochromatic images are formed on the exit slit.
4. Exit slit is the end of the optical journey in a monochromator. Many monochromatic images adjacent to each other are formed on a *focal plane*; one or more images are selected by exit slit(s).

The description above applies to a classical monochromator; the developed novel designs have deviations from this format. A monochromator is protected by dust, dirt or extraneous radiation by a tightly closed metal box; internal surfaces are coated with non-reflective, matt black paint. The terms *monochromator* and *polychromator* are often used in a loose manner; the latter is more proper for a system that is using more than one wavelengths selected at the exit slits at a time. A typical monochromator design is shown in Figure 5.18.

Alternatively, more than one wavelength, in practice up to ~60, may be selected simultaneously by using many slits and detectors; a popular polychromator design, *Rowland Circle*, is shown in Figure 5.19. It must be noted that in this design approach, no collimators are used since the concave grating is able to both disperse the light and form the image.

The images at consecutive wavelengths are ordered on a plane perpendicular to the optical axis; this is called the *focal plane*, as shown in Figure 5.18, extending from the points f to p. By rotating the grating, the conditions for the grating formula is met for another wavelength, so that the corresponding monochromatic band is allowed to reach the exit slit. The bandwidth of monochromatic radiation does depend on the width of the exit slit. Narrower slits result in smaller bandwidths for the monochromatic image at the exit slit. For a dispersion element forming monochromatic images, *resolution* is the measure of its ability in forming adjacent images

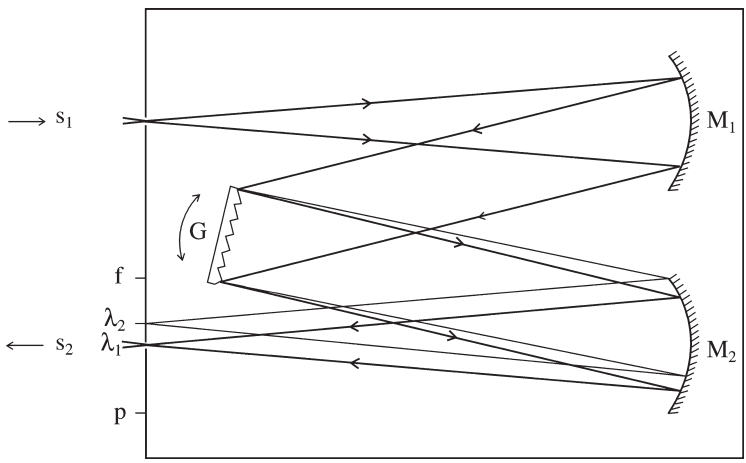


Figure 5.18 *A Czerny–Turner monochromator. S_1 and S_2 are the entrance and exit slits; G is reflection grating; M_1 and M_2 are off-axis mirrors used as collimators. Until G, white light is shown, after G the light shown is monochromatic; focal plane is from f to p*

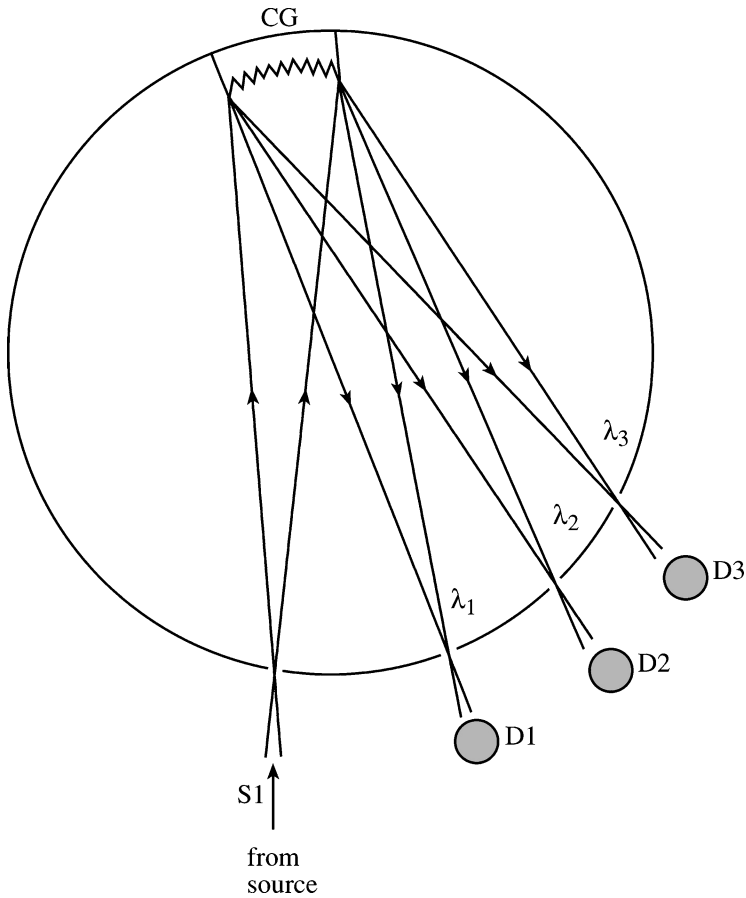


Figure 5.19 *A polychromator of the Rowland Circle type; S1, entrance slit; CG, concave grating; D1, D2, D3, detectors*

with wavelength differences as small as possible. Therefore both *dispersion* and *resolution* are the performance characteristics for a monochromator; while the former is a rather intrinsic property of the system, the latter is a measure of performance and is meaningful when considered with a defined slit width only.

Angular dispersion is given by dr/dλ, where dr is the change in the angle of reflection for a grating and refraction for a prism, as shown in Figures 5.16 and 5.17. The *linear dispersion*, D, is equal to dy/dλ or Fdr/dλ, where F is the focal length of a monochromator and dy is the physical displacement on the focal plane for dλ, the wavelength difference as shown in Figure 5.20. F corresponds to the focal length of the off-axis collimator mirrors and is also called as *the focal length of the monochromator*. The more common expression for the dispersion ability is the *reciprocal linear dispersion*, D^{-1}:

$$D^{-1} = \mathrm{d}\lambda/\mathrm{d}y = (1/F)\,\mathrm{d}\lambda/\mathrm{d}r \tag{5.18}$$

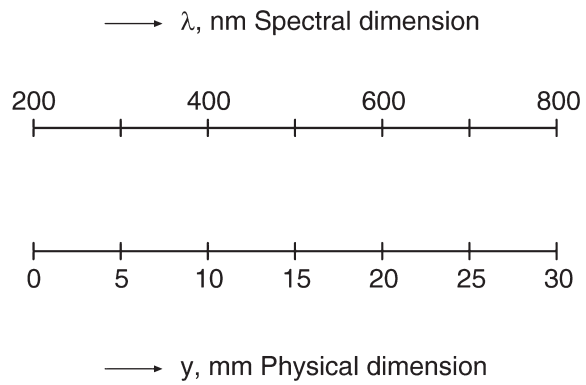


Figure 5.20 *Wavelength dispersion along a focal plane*

Common units for D^{-1} are nm/mm and this figure of merit is mentioned to express the intrinsic dispersion ability for a monochromator.

The angular dispersion of a grating can be found by differentiating the grating formula while i is held as a constant. The following expression is obtained for a given angle of incidence, i:

$$\mathrm{d}r/\mathrm{d}\lambda = N/(d \cos r) \tag{5.19}$$

It can be shown that

$$D^{-1} = (1/F)\, \mathrm{d}\lambda/\mathrm{d}r = (d \cos r)/NF \tag{5.20}$$

Resolution of a monochromator is defined in several ways. When λ is an average of two wavelengths that can form separate adjacent images on the focal plane and Δλ is their difference, *resolving power, R* is given as follows:

$$R = \lambda/\Delta\lambda = NN' \tag{5.21}$$

where N is the diffraction order and N' the number of lines illuminated on the grating surface. N' is a function of the monochromator design and the width of the grating. While the above equation is the definition for resolving power R, most users prefer to employ D^{-1}, reciprocal linear dispersion, or spectral bandwidth to express resolution performance of a system.

Slits are formed by placing two sharp metal edges parallel to each other. Optical requirements usually force that equal sizes for the entrance and exit slits must be used. While the entrance slit allows a certain portion of the radiation coming from the object, the exit slit defines the portion of focal plane images to be sent to detector. Therefore, large slits would allow higher number of photons on detector and this is advantageous to have a stronger light signal; on the other hand, a high wavelength resolution requires smaller slits that cause the degradation of signal power. When the two slit widths are identical, the slit function at exit slit is a triangle whose height is proportional to light power and the base has the units of wavelength, as shown in

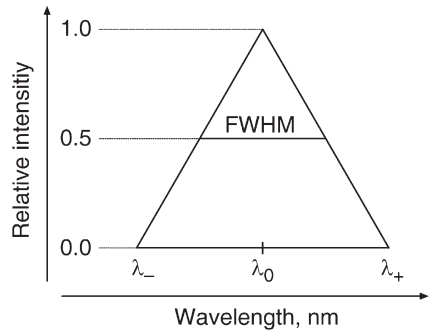


Figure 5.21 *Slit function*

Figure 5.21. *Effective bandwidth* is the half-width or FWHM value of this triangle in units of wavelength. When combined with the reciprocal linear dispersion, a useful relation is formed as follows:

$$\Delta\lambda_{\text{eff}} = WD^{-1} \tag{5.22}$$

where $\Delta\lambda_{\text{eff}}$ is the effective bandwidth or spectral bandwidth in nm, D^{-1} the spectral bandwith in nm/mm and W the physical slit in mm. In order to have a complete separation of two images at λ_1 and λ_2, $\Delta\lambda_{\text{eff}}$ should be equal to the half of the difference between these wavelengths.

Another important property of a monochromator is its *light-gathering power*, given by the *f/number* of the system,

$$f = F/d \tag{5.23}$$

where F is the focal length and d the diameter of aperture; d may correspond to the diameter of a collimator mirror or a lens provided that all of its surface is employed. By convention, a system having a focal length of 25 cm and a mirror diameter of 2.5 cm would have a light-gathering power equivalent to $f/10$; smaller $f/$numbers correspond to higher light-gathering powers. However, high resolution requires smaller light-gathering powers because rays are more paraxial and the images are sharper in such systems.

In order to have a high spectral resolving power, a value as small as possible is required for D^{-1}. Two different approaches have been used for this purpose.

- Classical grating monochromators use very fine rulings, such as 1200–3600 lines mm^{-1}, corresponding to a very small value for d; in addition, the use of a large monochromator with a high F value is advantageous. Normally, the orders of 1 or 2 are used. The angle r is rather small and the equation for D^{-1} practically becomes, in most cases, $D^{-1} = d/NF$.
- *Echelle monochromator* design has a different approach than the classical ones. A relatively high order, such as 40–135, is used; in addition, the angle of reflection, r is large and thus cos r is minimized. A rather coarse grating is used with

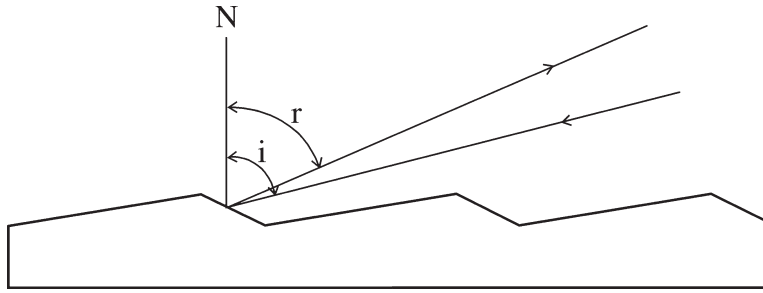


Figure 5.22 *Diffraction on an Echelle grating; N, normal; i, angle of incidence; r, angle of reflection*

Table 5.1 *Comparison for a conventional and Echelle monochromator*

	Conventional	*Echelle*
Focal length (m)	0.5	0.5
Groove density (lines mm^{-1})	1200	79
Angle of diffraction, *r*	10° 22′	63° 26′
Width of grating (mm)	52	128
Order *N* at 300 nm	1	75
Resolution at 300 nm	62 400	763 000
Linear dispersion at 300 nm (mm nm^{-1})	0.61	6.65
Reciprocal linear dispersion at 300 nm (nm mm^{-1})	1.6	0.15
f/number	*f*/9.8	*f*/8.8

typically 79 lines mm^{-1} and in contrast to conventional gratings the smaller face of the groove is used to receive and reflect the light as shown in Figure 5.22.

A comparison of performances for the two monochromator designs of the same focal length is given in Table 5.1.

Relatively high orders used for an Echelle monochromator create problems regarding the overlapping wavelengths at different orders at the same point on the focal plane. The overlap due to orders is a problem also with the conventional monochromators. However, *free spectral range*, $\Delta\lambda_{\text{eff}} = \lambda/(N+1)$, is rather large for small orders. For example, at $N = 1$ and 600 nm, free spectral range is 300 nm, meaning that there are no overlapping orders between 600 and 300 nm. Cut-off filters or wide-band filters are used as *order sorters* in conventional monochromators. However, for Echelle monochromators, high *N* values are used and thus the free spectral range is very narrow. This problem is alleviated by using a prism as an order-sorter after the grating as shown in Figure 5.23. The prism in the Echelle monochromator disperses the light already dispersed by the grating. However, the plane of dispersion by prism is perpendicular to the plane of dispersion by the grating. Therefore, while in a conventional monochromator the focal plane lies on one

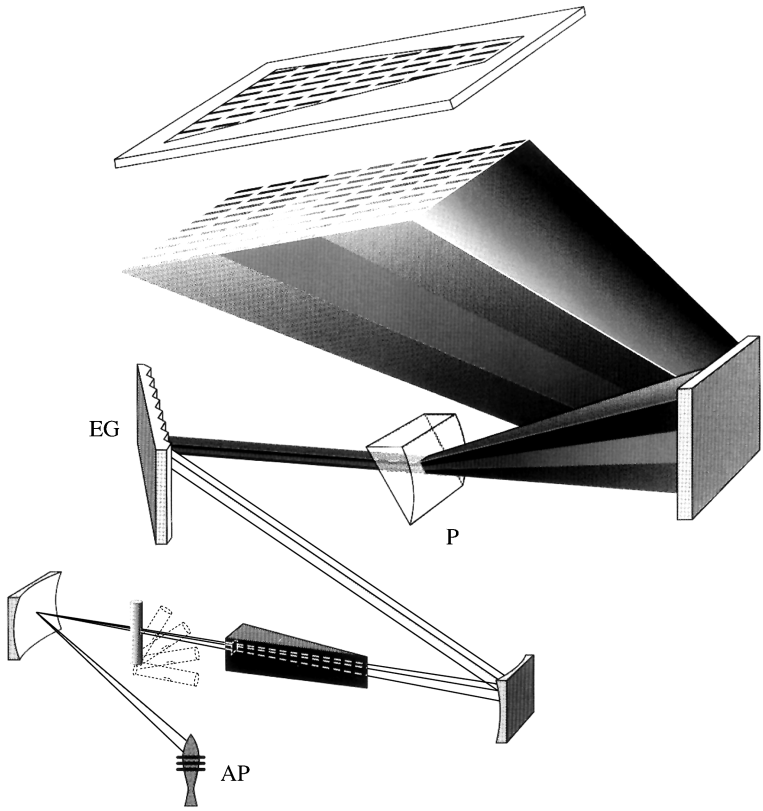


Figure 5.23 *A direct reading Echelle spectrometer; AP, argon plasma; EG, Echelle grating; P, prism (Adapted by permission from Teledyne Leeman Labs, USA)*

dimension, a *two-dimensional focal plane* is formed in an Echelle monochromator. The two-dimensional focal plane can accommodate single or multiple detectors as required. Both conventional and Echelle monochromators are used in several spectrometer designs in atomic spectrometry where high resolution is required, especially in emission measurements.

5.3.1.3 Sources

The term *source* may have different meanings in spectrochemistry. For all the techniques based on absorption, the source is a lamp whose radiation provides the photons to be absorbed by analyte. Source, on the other hand, for emission techniques is a high-temperature and/or high-energy system, which contains the analyte species in excited state, such as a flame, arc, spark or plasma. The features of absorption spectra of analytes and the emission spectra of sources used in these measurements have similarities. Molecular absorption spectra consisting of bands with rather large bandwidths require the so-called *continuum sources*. Tungsten incandescence lamps are

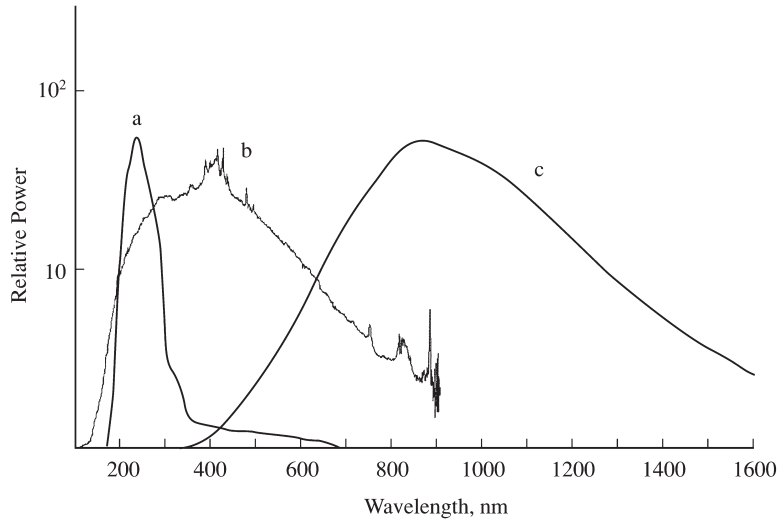


Figure 5.24 *Emission spectra of some commonly used sources in molecular absorption spectrometry; a, deuterium arc lamp; b, Xe arc lamp; c, tungsten lamp*

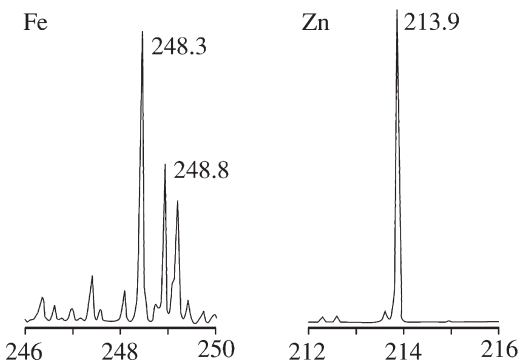


Figure 5.25 *Emission line spectra of Zn and Fe hollow cathode lamps*

inexpensive blackbody radiators to be used in VIS, while D_2 and H_2 lamps provide a continuum in UV region. Xe lamps have continuous radiation in both UV and VIS regions. Some of these lamps and their emission spectra are shown in Figure 5.24.

In contrast to continuum sources used in molecular absorption, *line sources* are employed in atomic spectrometry. Emission line spectra of some hollow cathode lamps are shown in Figure 5.25. The natural bandwidths of atomic emission lines are about 10^{-5} nm; with broadening effects they become as high as 10^{-3} nm. If the slit function, as shown in Figure 5.21, is considered at any selected wavelength, this triangular function is to be multiplied with the emission spectrum of the source for the wavelength interval defined by the spectral bandwidth.

The result of this multiplication resembles the slit function for a continuum source, where typically 0.5–5.0 nm spectral bandwidths are used for molecular

measurements; therefore the limiting factor for resolution is the physical slit size W and reciprocal linear dispersion D^{-1}. On the other hand, for atomic absorption spectrometry, spectral bandwidths used are in the range of 0.1–2.0 nm; since these values are much smaller than atomic emission linewidths, *e.g.* 10^{-3} nm, the multiplication of the slit function with the emission profile of the line lamp resembles the emission profile and not the slit function. Therefore, the limiting factor for resolution is the emission line bandwidth of the source and not the monochromator parameters. In order to have a satisfactory resolution for molecular absorption spectrometry, monochromator bandwidth should be less than or equal to 1/10 of the bandwidth of the absorption peak under study. The nature of atomic line sources for absorption and emission sources will be discussed in their respective chapters.

Infrared spectrometry employs blackbody sources, such as a *nichrome wires* (Ni+Cr), *Globar* (SiC) and *Nernst Glower* ($ZrO_2 + Y_2O_3$).

Laser is a special kind of light source, having differences from the others in many respects. The name *laser* is an abbreviation for "light amplification by stimulated emission of radiation". In other light sources, photons emitted do not have any defined phase relation; therefore many photons, for example from a tungsten filament lamp, may be lost because undefined phase relations will partly result in a totally or partially destructive interference. Lasers, on the other hand, have an optical and electronic structure and nature, which are designed to produce photons of same wavelength and constant phase relations. These well-defined phase relations are such that maximum constructive interference is assured; in other words no photons are wasted by destructive interference. As a result, a laser source having a power of few mW can provide a light intensity equivalent to a continuum lamp of hundreds of Watts. Laser sources have the following distinct properties.

- Monochromatic radiation, bandwidths are 0.01 nm or less.
- Plane polarized radiation.
- Very low beam divergence, 0.05° or less. Therefore a laser beam stays thin over long distances of travel.
- Spatially very narrow (<1 μm) and very intense light beams.
- Highly *coherent* beams, as a result of constant phase relations among the waves of same wavelength.
- Available in regions of UV, VIS and IR. Examples for extreme ends are F_2 laser, (152 nm) and CO_2 laser (10.6 μm).
- For spectrometric uses, lasers are available with powers as low as 0.1 mW and as high as 500 mW.
- Lasers can be used in pulses with durations as short as 10^{-15} s.

Despite their extraordinary properties, use of lasers as light sources is limited. One important disadvantage is the availability of only few wavelengths from a single source. Tunable dye lasers offer scanning ability for a limited range of 20–50 nm. Another negative aspect is that most lasers are noisier than classical continuum lamps; therefore their use in absorption spectrometry is not common.

The uses of lasers in spectrometry are limited to light sources in Raman spectrometry for molecules and sources used to vaporize and/or to excite samples in

Table 5.2 *Some laser sources used in spectrometry*

Laser type	*Name*	*Properties*
Gas	He–Ne	543.5, 593.9, 611.8, 632.8, 1523.1 nm, few mW
	Ar–ion	457.9, 488.0, 514.5 nm, high power
	Kr–ion	415.4, 520.8, 647.1, 676.4 nm, high power
	CO_2	10.6 μm, pulse widths of 1–200 μs
	Nitrogen	337 nm, pulse width of 300 ps, repetition rates of 1–100 Hz
	Excimer	ArF (193 nm), XeF (351 nm), KrF (248 nm), XeCl (308 nm), repetition rates up to 250 Hz, pulse widths of 7–20 ns.
Liquid	Dyes	330–1000 nm, each dye is tunable in a range of 20–50 nm, pulse widths of 100–300 Hz.
Semiconductor diodes	—	630–1600 nm, pulse widths of 0.1–100 ns
Solid state	Ruby	694.3 nm, high power pulses of 10 J
	Nd-YAG*	1064 nm, 1320 nm, high-power pulses of 1–2 J.

*Yttrium aluminum garnet

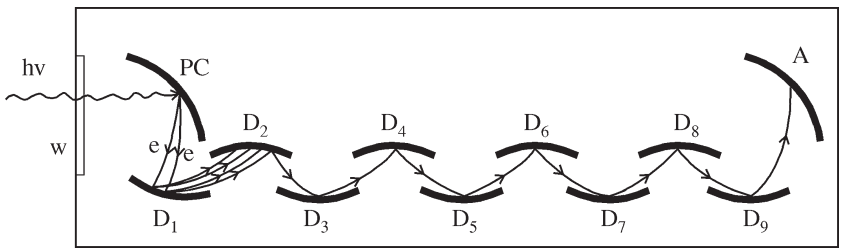


Figure 5.26 *A photomultiplier light detector; w, window; PC, photocathode; D1–D9, dynodes; A, anode; hv, photon; e, electron*

atomic fluorescence spectrometry. Probably, the most frequent use of lasers in atomic spectrometry is *laser ablation* technique; this is now highly commercialized. In this technique, the laser falling on solid sample surface ablates the sample, an aerosol consisting of free atoms, volatilized species and solid particles with a size of less than 5 μm is formed. This mixture can be transported by using a carrier gas such as Ar, into an Ar plasma where the subsequently formed species can then be determined by ICP-OES or ICP-MS. Some typical lasers are given in Table 5.2.

5.3.1.4 Detectors

Detectors used in spectrometry are transducers, which are able to convert light energy into electrical energy. In UV–VIS region, *photoelectric detectors* are commonly used. The most sensitive and popular photoelectric light detector has been the *photomultiplier tube*, commonly abbreviated as PMT. This detector, although still being used for

many devices, is now being replaced by array detectors in some instrument designs. The operation principle of a PMT detector is shown in Figure 5.26. A PMT detector contains a cathode, an anode and typically 9–11 dynodes in vacuum; the system has a transparent quartz envelope. The cathode is made from a material having a *photoemissive surface*, photons striking the cathode cause the ejection of photoelectrons that travel to the first dynode, which is held at a potential of approximately 90 V dc more positive than the cathode. The other dynodes in series and finally the anode are all held at successively higher positive potentials. Electrons arriving at the first dynode cause the ejection of 3-5 other electrons that will travel to the second dynode that is held at a more positive potential; consequently after passing through some number of dynodes, a large number of electrons will reach the anode at the end. The current between the anode and the cathode is measured and above a threshold value for the applied voltage, this current is linearly correlated to the number of photons or the power or radiation.

PMTs are presently not only the most sensitive light detectors, but they also have the largest dynamic range as high as six orders of magnitude. This property is very important for their use with analytical techniques having inherently large dynamic ranges. Photocathode materials are photoemissive surfaces usually consisting of alkali metals in various combinations and mixtures to give relatively low values for work function, W. According to the principles of photoelectric effect, the energy of the oncoming photon, $h\nu$, should be at least as high as the work function W; any excess in the light energy is transferred to the ejected electron as its kinetic energy. The work function of photoemissive surfaces is never as low as the energy of the IR photons; therefore photoelectric detectors cannot be used in this spectral region. The cathodes can work on transmission or reflection modes. The most employed photoemissive surfaces are Ag–O–Cs and Sb–Cs for UV and VIS, bialkali (Sb–Rb–Cs and Sb–K–Cs) for UV and VIS, high-temperature bialkali (Na_2.K.Sb), multialkali (Na–K–Sb–Cs) with UV–VIS response extended to near IR, wide range Ga–As–Cs with a flat UV–VIS response and Cs–Te or Cs–I for solar blind (only UV) type of responses. Some examples for photoemissive surface responses are given in Figure 5.27.

Advances in electronics technology and science have resulted in relatively novel products as strong alternatives to PMTs; these are *array detectors.* Small light detectors with sizes in micrometre scale are now available. A classical, scanning monochromator such as the one shown in Figure 5.18 has an exit slit and a single detector to receive the selected portion of wavelength from the focal plane. In alternative monochromator designs, there is no exit slit; grating and thus the focal plane is stationary. A large number of individual, small detectors are located in an array on the focal plane; each detector receives an allocated portion of the spectrum. Such systems are called *photo diode array (*PDA*)* detectors. Since light signals of all the wavelengths are measured simultaneously, the whole spectrum can be obtained in periods in the order of a second. The individual detector size limits the spectral resolution. The sample has to be placed before the entrance slit, after the source. Since the period of exposure for the sample is very short, photodecomposition of molecules in measurement zone is not likely for UV–VIS molecular spectrometry. In addition, any stray light induced in sample cuvette

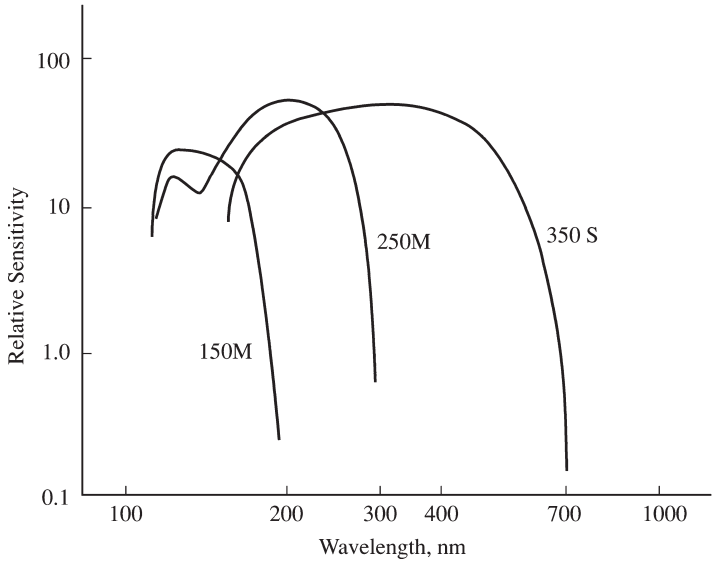


Figure 5.27 *Response curves for several photoemissive surfaces (Reproduced with permission from Hamamatsu Photonics K. K., Japan)*

will be eliminated by the wavelength selector, resulting in improved linearity. PDA systems are very popular for molecular spectrometry, especially with spectrometric detection used as coupled with chromatographic separation. Using a computer for data acquisition, three-dimensional plots can be easily stored; the information thus obtained contains the change of signal with both time and wavelength. Therefore chromatograms at any wavelength and spectra at any elution time can be obtained by using the selected data. The information obtained is very rich.

Photodiodes are usually silicon diodes; radiation falling on the detector surface alters the charge characteristics, finally a recharging current is measured and correlated with the quantity of radiation received. The individual photodiode detector size is typically 20–25 μm, this is analogous to an exit slit size. The working range is typically 190–1100 nm. The use of PDA systems is very common in molecular UV–VIS spectrometry.

Array detectors are also used widely in atomic spectrometers. In many atomic emission spectrometers two-dimensional arrays of *charge transfer devices (*CTD*)* are employed with Echelle polychromators and the two-dimensional focal plane formed therein, such as shown in Figure 5.23. The operating principle of CTDs is somewhat similar to that of photodiodes; however these devices can be used in two-dimensional arrays. There are different kinds, such as *charge coupled device (*CCD*)* and *charge injection device (*CID*)*. Working range is from UV to near IR. The surface area of a single CTD is commonly 15–30 μm squared.

Regarding performance characteristics, PDAs have the lowest sensitivity, PMTs are best and CTDs can now compete with PMTs but require usually longer integration times.

Further Reading

1. J.D. Ingle Jr. and S.R. Crouch, *Spectrochemical Analysis*, Prentice-Hall, New Jersey, 1988.
2. D.A. Skoog, F.J. Holler and T.A. Nieman, *Principles of Instrumental Analysis*, 5th edn, Saunders College Publishing, Philadelphia, 1998.
3. G. Currell, *Analytical Instrumentation*, *Performance Characteristics and Quality*, Wiley, Chicester, 2000.
4. K.A. Rubinson and J.F. Rubinson, *Contemporary Instrumental Analysis*, Prentice-Hall, Upper Saddle River, NJ, 2000.

CHAPTER 6

Atomic Absorption Spectrometry

6.1 Introduction, History and Principles

Although the use of atomic emission has been in effect for almost two centuries, employment of the concept of atomic absorption is relatively new. The first articles on *atomic absorption spectrometry (AAS)* were published by Walsh[1] in Australia and by Alkemade and Milatz[2,3] in The Netherlands in 1955. The atomic emission spectrometry (AES) is based on the radiation emitted by thermally excited atoms as they relax to the ground-state; AAS relies on the absorption of resonance radiation by the analyte atoms at their ground-state. Atomization of the sample is required in both AES and AAS; one of the most convenient ways has been the use of a flame as an atomizer in which a liquid sample is aspirated into this atomizer to form the free, ground-state atoms. In this atomized state, the ground-state atoms are the analyte species for AAS. Following an era by flame AAS, graphite furnace atomization was introduced by L'vov[4]; in this system a resistively heated carbon cuvette was used as atomizer; the idea was somehow based on the work of King.[5] Although L'vov's device was very interesting and effective, a modified form of this heated graphite atomizer suggested by Massmann[6] was commercially manufactured; a similar design was also suggested by Woodriff.[7] The graphite atomizer was more sensitive but also more prone to chemical and spectral interferences as compared to the flame atomizer. Since similar atomizers were made from materials other than graphite, the common name, *electrothermal AAS (ETAAS)*, has been adopted for this technique.

Other important milestones in the history of AAS are related to the developments regarding background correction, which became an important problem particularly associated with ETAAS. These were the inventions of background correction techniques employing a continuum source by Koirtyohann,[8] Zeeman splitting[9] and self-reversal of emission lines by Smith and Hieftje.[10] AAS is basically a single-element technique, since a line source for each element is required. However, there has been a continuous effort to design a multi-element AAS system; these works include the use of a single continuum source and a high-resolution Echelle monochromator[11]; despite some drawbacks, this approach has been quite successful but it could not become a part of routine applications in laboratories for a long time. Recently, an AA instrument with a single continuum Xe source and computer-controlled background correction has been manufactured.

Among the other atomization techniques for AAS are *hydride generation AAS (HGAAS)* for elements such as As, Se, Sb, *etc*., cold vapour formation for Hg, several atom trapping devices using flame, Pt-loop and W-coil atomizers and others aiming higher sensitivity at lower cost. These approaches will be discussed in more depth later in this chapter. Both flame and ETAAS are techniques for samples mostly in solution form. However, there have been studies for introducing solid samples or slurries into atomizers, as will be discussed later.

In an AAS measurement, absorption of resonance light energy by ground-state free atoms is correlated to analyte concentration. Thermal energy is employed to desolvate, dry and atomize the analyte species. The transitions are only electronic since the chemical bonds are virtually all broken and only UV and VIS regions are involved. The Boltzmann equation illustrates that in AAS atomizers using a maximum temperature of about 3000 K, a large majority of atoms are at the ground-state, forming the analyte species ready to absorb the source radiation. The spectral profile of atoms to absorb the radiation have a bandwidth of about 0.001 nm, which is smaller than the low-cost monochromator bandwidths employed, typically around 1.0 nm. Therefore, the absorbance value to be obtained by using a continuum source is obviously very small; a line source must be used for high sensitivity. The correct approach of using a line source was one of the major novelties suggested by Walsh[1] for his first AAS instrument. A sensitive signal by a continuum source could be obtained only by employing a high-cost monochromator with a bandwidth as small as that of the absorption profile, as used in continuum source multi-element AAS studies.[11] Absorption by analyte atoms using line and continuum sources are illustrated in Figure 6.1. Since the absorbance value obtained when a continuum source and low-resolution monochromator used is rather low, line sources are commonly used in AAS; the spectral resolution is limited by the source bandwidths rather than the monochromator spectral slits, as in contrast to molecular absorption measurements. Please note that the illustrations in Figure 6.1 and some other figures in this chapter (Figures 6.12–6.14 and 6.17) show *a section of the scanned spectrum*. Otherwise, all the intensities near both the ends of the spectral slit would approach to zero, since the slit function is a triangle. This style was preferred to better emphasize the contrast between a line source and a continuum source.

The relation between the signal and analyte concentration in an AAS measurement obeys Beer's law.

$$A = kC = \log (P_0/P) \tag{6.1}$$

where P_0 is the power of incident radiation, P the power of radiation after absorption, C the analyte concentration and k a constant; the relation is valid at the selected wavelength. The Beer's law can be used for AAS; however, the situation is somewhat different as compared to the molecular absorption measurements:

- In molecular absorption measurements using sample solutions, analyte species having the concentration C and the incident radiation with the power P_0 has a direct interaction in the sample cell. In AAS measurements, however, only a fraction of C is made available in the atomizer. Since AAS is a comparison

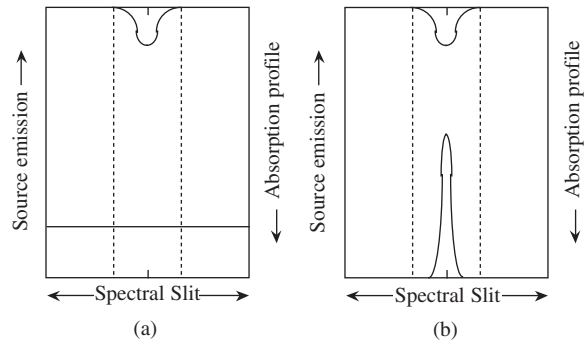


Figure 6.1 *Absorption by analyte atoms at resonance wavelength using (a) continuum source and (b) line source*

technique, it is essential that the same efficiency must be valid for the sample and standard solutions regarding the transport of a portion of analyte into the atomizer in the form of ground-state atoms. Therefore, in practice, the value of k includes this efficiency as well as the probability for absorption and the pathlength.

- In contrast to a solution in a sample cell, the ground-state atoms in an AAS atomizer do not constitute a homogeneous mixture. The pathlength b must not be incorrectly interpreted. Most atomizers contain atoms in higher density in the central region as compared to the flanks. For example, doubling the pathlength of a flame atomizer will result in an increase in absorbance amounting to less than twice.

6.2 Instrumentation

6.2.1 Sources

In Section 6.1, it was demonstrated why a line source is required for AAS. The most popular line source is the *hollow cathode lamp (HCL).* It is in the form of a glass cylinder containing Ne or Ar as inert gas, a cathode containing the analyte in form of pure metal or its alloy and an anode, as shown in Figure 6.2.

The cathode is shaped as a hollow cylinder; the nickel or tungsten anode is usually a wire near the cathode or a ring above it. The pressure in the device is a few Torrs. Several hundred volts are applied between the electrodes and a discharge takes place almost completely within the cathode. The current applied varies between 5 and 30 mA and should be optimized to give the best intensity without causing any line-reversal effects. A stream of positive ions of the fill gas strikes the surface of cathode material and the analyte atoms are released by an action called *sputtering*. These atoms further interact with the fill gas ions and excited atoms; excitation of analyte atoms takes place to radiate at their specific wavelengths as they relax back to the ground-state. Some HCL devices are multi-element; the cathode contains the material to include several analyte elements, typically 2–3. Multi-element HCL devices

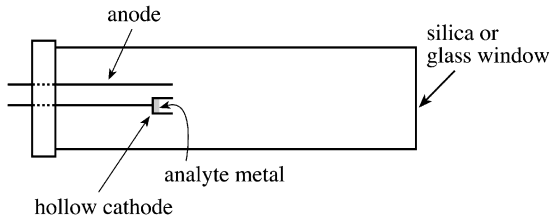


Figure 6.2 *Hollow cathode lamp*

usually suffer from lower intensity per analyte as compared to the single-element lamps. High-intensity HCLs use a slightly different design; a second cathode is included, which provides a secondary discharge through the primary cathode. An auxiliary power supply is needed to operate the system at higher power in addition to the conventional power supply of commercial spectrometers. There are several reasons for shortened HCL lifetime. The fill gas may be adsorbed on the walls, the pressure drop of these gases result in lower efficiency of sputtering and thus lowered intensity; some manufacturers suggest that a larger volume for HCL is advantageous as this design will be more tolerant to the decreases in the fill gas pressure. Obtaining the pure metal of analyte is difficult; during sputtering, the hydrogen gas trapped in the metal may be released and cause spectral interferences; a tantalum getter on the anode absorbs this gas and minimizes these problems.

The line radiation supplied from an HCL should be narrower than the absorption profile of the analyte atoms in the atomizer; otherwise the unabsorbed radiation at the wings cause a non-linearity in signal with increasing analyte concentration.

Electrodeless Discharge Lamp (EDL) is another important radiation source for AAS. These devices provide high intensity and narrow linewidths. The system is enclosed in an evacuated quartz envelope; the fill gas has a pressure of few Torrs. Analyte is placed into a quartz container as its metal or its compound. A radiofrequency coil of about 27 MHz is used to form a vapour of excited analyte atoms. EDL devices are used for elements such as Sb, As, Bi, Cd, Cs, Ge, Pb, Hg, P, K, Rb, Se, Te, Tl, Sn, Ti and Zn. They require a separate power supply and their cost is somewhat higher as compared to HCL devices. Highest intensity and longer lifetimes are advantages.

In the early stages of AAS, flames were also used as radiation sources by Alkemade[3]; high concentrations of analyte salts were aspirated into the flames, which would then become atomic emitters. One important advantage for these sources was that one did not have to obtain the pure metal or an alloy for analyte element; any compound could be used in solution form. However, these types of sources did not become commercialized. The emission linewidths were even broader than that of absorption profiles, resulting in a non-linear performance. In addition, the flame emission sources were not very stable.

As mentioned before, continuum sources could be employed only when monochromator bandwidths are as narrow as the linewidths of an emission line. Continuum source AAS instruments were designed by Zander *et al.*,[12] where a 200 W Xe arc lamp or a 150 W Eimac® lamp was used as a source with a high-resolution Echelle

monochromator, and by O'Haver *et al.*[13] using a similar system and a 300 W Eimac® lamp. The studies still continue[14] as these instruments offer the distinct advantage of providing multi-element determination.

6.2.2 Monochromators

AAS instruments employ rather low-resolution monochromators having a focal length of 20–35 cm; typical spectral slits vary between 0.1 and 2.0 nm. In conventional single-element AA spectrometers, the resolution is limited by the emission linewidth of source, rather than the spectral slit. Multi-element AAS instruments using continuum source employ spectral bandwidths as low as 0.01 nm by using Echelle monochromators; in these research level systems, the resolution is defined by the wavelength dispersing device.

6.2.3 Atomizers

An ideal atomizer for AAS should have the following characteristics:

- Fraction of free analyte atoms in atomizer from those in the sample solution should be maximized. This requires an efficient transport of analyte from sample to the atomizer, as well as an efficient atomization in the atomizer.
- Once the atomic cloud is formed in the atomizer, the maximum physical overlap of the analyte with the source radiation must take place. This is a function of the geometrical and optical design of the instrument.
- The analyte atoms should stay in the volume that overlaps with the source beam as long as required, so that the analytical signal will have a maximum *S*/*N* ratio. This period is called *residence time.*

6.2.3.1 Flame Atomizers

The conventional atomizer for AAS is a laminar, pre-mixed-type flame. Laminar geometry is convenient because it provides a longer pathlength along which the analyte atoms and the source beam can have a good overlap to form the absorbance signal. The pathlength is commonly 5 or 10 cm. Although several types of flames were tested in literature, in our days only two of these are used effectively. Air–acetylene flame is the most popular one providing a maximum temperature of 2300 °C. This temperature is not sufficient to break some metal–oxygen bonds for a number of elements such as Si, Al, W and Ti. In these cases, the atomizer is important in atomization process, it should be remembered that the N_2O–acetylene flame is used; its maximum temperature is 3000 °C. Although the temperature itself is far from being chemically inert. Therefore, it has been suggested that the chemical environment in flame may also be important regarding the atomization process.[15,16] Indeed, fuel/oxidant ratio of a flame has a great effect on atomization signal. The flame composition is given several names reflecting its fuel/oxidant ratio. A *stoichiometric flame* is the one having a stoichiometric ratio for the fuel and the oxidant, none of them is in

excess. A *fuel-lean* flame has a fuel/oxidant ratio less than unity; it is used effectively for atoms that do not easily form oxides in the flame having excess of the oxidant gas, such as Cu, Pb, Cd, Zn, *etc*. On the other hand, a *fuel-rich flame* is a reducing flame and it contains the fuel in excess; its typical use is for the determination of Cr, which is easily oxidized in a fuel-lean flame. For each element, the best flame composition is suggested regarding its type and the fuel/oxidant ratio. In addition, the location of the free atoms in a flame atomizer varies for each element; a geometrical optimization is necessary to obtain the greatest overlap of the free atoms and the source beam.

The physical transport of sample solution into the flame is realized by the vacuum formed as the fuel and oxidant gases are introduced into the mixing chamber. The solution is sucked into this vacuum, at a rate of typically 3–6 mL min^{-1} through a *nebulizer* that is basically a fine needle. The drops of sample are reduced in size by a high-pressure gas flow perpendicular to the axis of the solution transport. As the droplets are sucked towards the flame, further barriers such as a sphere (*impact bead*) and/or some blades (*flow spoilers*) on the way also help reduction of drop sizes so that finally a mist arrives the flame. The majority of sample solution is drained to the waste. The analyte atoms in the flame are in a dynamic equilibrium; the constant atomic absorption signal is based on the quantity of atoms present per unit time. This signal persists as long as the sample is introduced into the flame, as shown in Figure 6.3. Once in the flame, sample droplets go through several stages until the formation of free ground-state atoms is achieved, as shown in Figure 6.4. It is necessary to alter fuel/oxidant ratio without upsetting the nebulization process; therefore, an auxiliary oxidant flow is supplied into the mixing chamber as well as the fuel and normal oxidant gas channels.

The fraction of analyte reaching to the flame is expressed as *nebulization efficiency*, which is between 1 and 10%. On the other hand, the average residence time

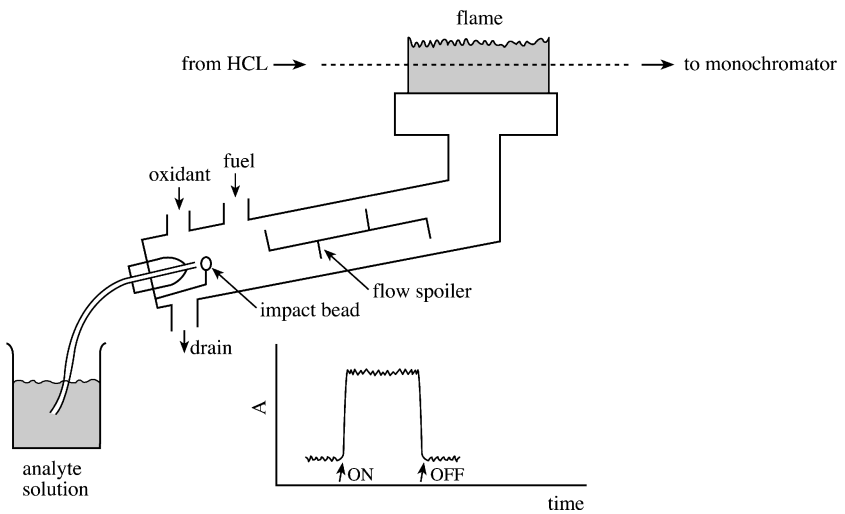


Figure 6.3 *Schematic representation of a nebulizer–spray chamber–burner system and formation of the steady-state signal using a flame atomizer*

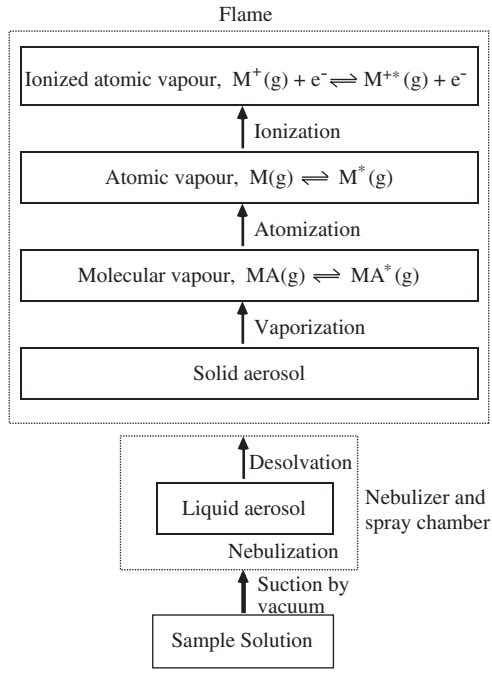


Figure 6.4 *The stages of analyte species in a flame atomizer*

of analyte atoms in the flame is about 0.1 ms. This rather short period of time together with the low nebulization efficiency are the main disadvantages of flame atomizer, limiting its sensitivity to mg L^{-1} range. In addition, at least few millilitres are required for sampling; for some applications, this volume is high as compared to the available amount of the sample. Although there are studies for spraying solid samples in the form of dispersions into flames, the flame atomizer is basically limited to solutions. The necessity of employing flammable gases is another disadvantage regarding the laboratory safety and automation as continuous attention is needed. Despite these disadvantages, flame atomizers are easy to use and the cost of analysis is relatively low. Because of low nebulization efficiency, which was mentioned as a disadvantage above, the contents of the flame are diluted and interferences are low as compared to other more sensitive atomizers, such as graphite atomizers, which will be discussed later. As a result, high accuracy and precision figures better than 1% RSD are common with flame atomizers.

6.2.3.2 Furnace Atomizers

Furnace atomization is defined using several names such as *Graphite Furnace Atomic Absorption Spectrometry* (*GFAAS*) or *ETAAS*. In contrast to the flame atomizers in which the analyte atoms are in a dynamic equilibrium, in furnace atomizers the signal is caused by only the number of atoms derived from the sample placed in the furnace.

The furnace atomizer mainly consists of a graphite cuvette that is typically 3–5 cm long and 5–9 mm in diameter, as shown in Figure 6.5. The graphite cuvette is kept in an Ar or N_2 atmosphere to prevent burning of carbon. Ar is a better inert gas since in the presence of carbon N_2 will form CN radicals whose spectrum will cause spectral interferences. Graphite sublimes at around 3000 °C and thus can be heated up to this temperature in an inert environment. The cuvette is resistively heated by electricity. The protective jacket around the cuvette sustains the inert atmosphere; cooling of the system is provided by water circulation. 5–50 μL of sample solution is introduced through the sampling port either manually by a plastic-tip pipette or an automatic sampling device. The position of the drop in cuvette is important; the drop position should be reproducible. Recently, a system for observing this position has been developed using an optical monitoring device whose image can be displayed on the screen of a personal computer, as shown in Figure 6.6.

The heating of graphite cuvette can be programmed regarding the time and the temperature, allowing custom design of the steps required for a particular analysis. The following steps are commonly employed:

Drying. The sample is usually in a solution form. A temperature below the boiling point of solution is applied. The drying should be smooth; boiling should not take place. Otherwise, the contents may be scattered to different locations of the atomizer in each determination, and thus the precision and accuracy may are adversely affected. The drying regime may consist of a linear heating stage, a ramp or their mixture. The inert gas flow is maintained to remove the solvent vapour.

Ashing. This is very similar to the ashing step that is commonly performed in many analytical procedures requiring the destruction of matrix. The purpose of ashing is to oxidize and remove the organic content in the sample. The ashing temperature should be as high as possible to remove the maximum matrix content, and yet

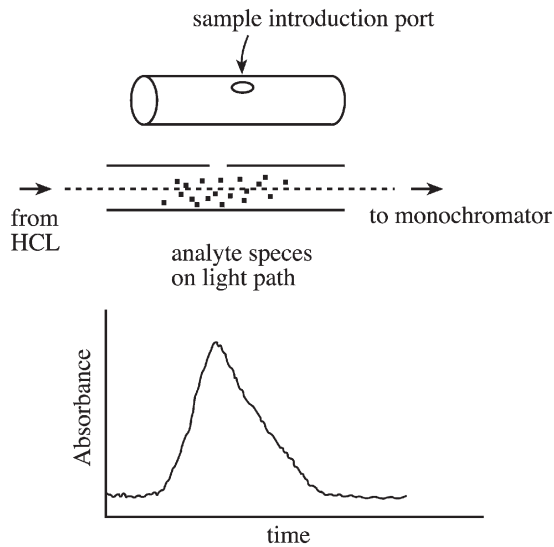


Figure 6.5 *Schematic representation of a graphite cuvette and signal formation*

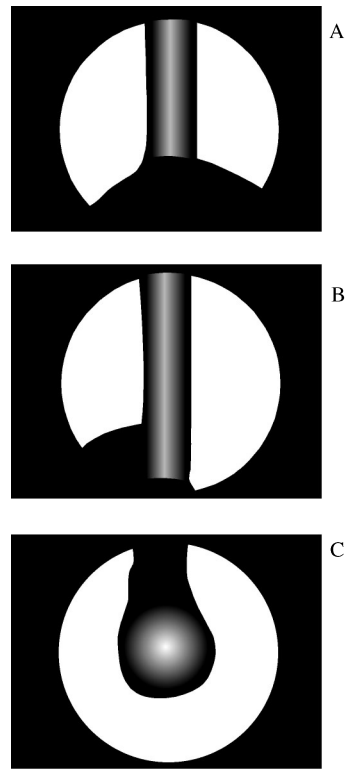


Figure 6.6 *Monitoring of the sample drop position in furnace atomizer using an optical device and personal computer; A, correct injection depth; B, capillary too low; C, capillary too high (Reproduced with permission from Thermo Electron Corporation, UK)*

low enough not to cause any analyte atomization. The ashing period commonly includes ramp heating so that the organic compounds are sequentially and smoothly removed without causing any explosions in the sample mass. Typically, temperatures ranging in 200–2000 °C with inert gas flow are employed for ashing. The heating regime may be designed by the performer as needed. An incomplete ashing will leave organic materials in the cuvette; this will possibly cause interference during the atomization stage. The presence of the organic molecules and decomposition products in cuvette during atomization cause a high extraneous background signal; this can be separately observed in most AA spectrometers.

Atomization. After the sample is cleaned from its organic content as much as possible, temperature of the system is suddenly increased to cause analyte atomization. Depending on the analyte, applied temperatures vary between 1200 and 3000 °C. Excessively high temperature adversely affects the cuvette life; in addition, the sensitivity may be lowered due to the rapid expansion of the inert gas transporting the analyte species out and minimizing the residence time. The atomization of analyte will include several steps from its solid compound to analyte atoms. During these intermediate stages, volatile oxides and/or halides of analyte may form. Owing to the increase in temperature, the inert gas will rapidly expand and will tend to diffuse out

of the cuvette together with the analyte species, leaving the volume in which the analytical signal is to be formed. Although the inert gas flow in the cuvette is usually interrupted during the atomization cycle, the rapid expansion of the contents may transport some of the analyte in unatomized form, out of the cuvette; this will cause a reduction in the analytical sensitivity. Therefore, if the rate of increase in temperature is faster than the diffusion rate of the volatile intermediate analyte species, an efficient atomization will take place; the intermediate species will still form, but without being able to diffuse out of the atomizer they will be decomposed to provide the analyte atoms. In modern graphite atomizers, a maximum power is applied for a rapid heating, as soon as the temperature of the cuvette reaches to the desired level, this is optically detected by graphite furnace emission and the power is leveled off. As the analyte atoms are formed and then diffuse out of the cuvette, a transient signal is formed and recorded, as shown in Figure 6.5. During atomization, inert gas flow may be continued or a reduced or mini-flow may be applied at the expense of sensitivity. The analytical signal may be the height of the peak in units of A, absorbance, or the peak area may be utilized using the units of A·s, absorbance times seconds.

Cleaning. The atomization temperature should be only as high to effect the analyte atomization, as mentioned above. However, the sample often includes other species that remains unvolatilized after the atomization stage. Therefore, in order to clean the cuvette prior to the next atomization, maximum temperature available for the system is applied for about 5 s accompanied by the inert gas flow. In order to have an efficient and reproducible atomization, the temperature programme for a furnace atomizer should be carefully optimized. A typical temperature programme is shown in the Figure 6.7.

It should be remembered that the instrumentation available today allows using as high as about 20 steps for a temperature programme; especially the drying and ashing steps may contain several linear and/or ramp heating regimes as required for the optimum conditions. When a sample with a new matrix is to be analyzed using ETAAS, it is customary to optimize the maximum ashing and the atomization temperatures as an important part of the temperature programme. This is often realized by using an *ash-atomize plot*, as shown by the Figure 6.8. Iterations may be necessary using this approach. Using a predetermined atomization temperature, the sample is ashed at varying temperatures prior to atomization. The recorded atomization signal starts to decrease if the ashing temperature employed becomes high enough to effect analyte atomization, leaving less analyte to the atomization step. For the other part of this plot, a predetermined ashing temperature is used and the atomization temperature is varied. Usually the minimum atomization temperature that will effect full atomization is selected for the reasons mentioned above. The reactions and the behaviours of analyte and interferants are complex in an atomizer; therefore, the comments to be made here should be taken only as general guidelines.

In most furnace designs, the cuvette is in contact with the graphite electrodes at both ends providing a *longitudinal heating*. In this case, similar to any other resistor, the graphite cuvette will have the highest temperature in the centre and will be gradually cooler towards the ends. Having a thermal gradient in the atomizer causes problems; for example, atoms formed in the centre may be condensed at the cooler

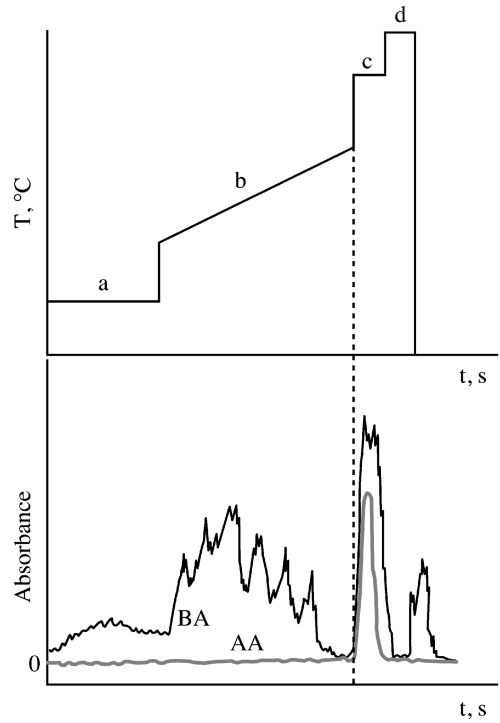


Figure 6.7 *A typical temperature programme for a furnace atomizer. a: drying; b: ashing; c: atomization; d: cleaning; AA: atomic absorption (corrected); BA: background absorption*

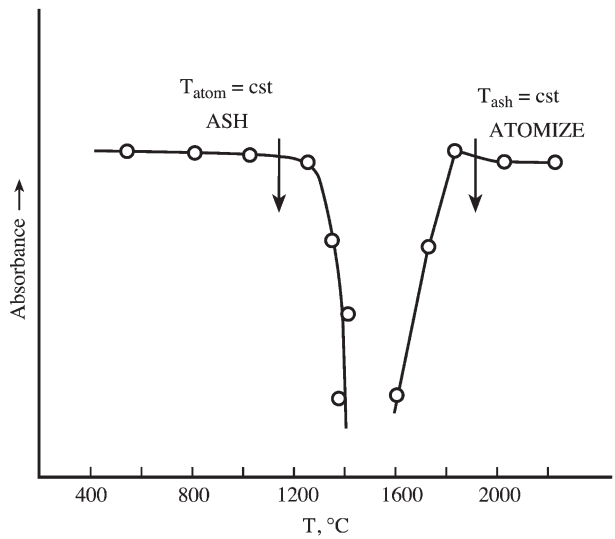


Figure 6.8 *Ash-atomize plots in a graphite furnace atomization*

ends. Recently, novel designs were made available where the cuvettes are heated not from the ends, but its sides providing a *transverse heating*. The principle of longitudinal and transverse heating with the temperature profiles along the cuvette is shown in Figure 6.9. Almost ideal *isothermal conditions* are obtained using transverse heating with a more uniform temperature distribution as compared to the longitudinally heated cuvette.

There are many other methods and approaches to improve the quality of a determination using ETAAS; these will be handled after the interferences and background correction methods are discussed.

6.2.3.3 Cold Vapour Atomic Absorption Spectrometry (CVAAS)

Alternative ways of atomization have been developed in order to improve sensitivity in AAS. In most of these techniques, the main idea is to obtain a concentrated atomic cloud to be detected; separation from the matrix constituents is also often accomplished.

Formation of an atomic cloud is normally realized by placing analyte species in a hot environment such as a flame or a furnace, as high temperature is necessary for both forming and sustaining the free atoms. One important exception is mercury that can exist as free atoms at ambient temperatures. CVAAS has been developed as an analytical technique for total Hg determinations.[17,18] Analyte species are converted to $H^{2+}(aq)$ by appropriate decomposition techniques using reagents such as nitric acid, sulfuric acid and potassium dichromate; a strong reductant containing $SnCl_2$ or $NaBH_4$ is then added to form gaseous mercury:

$$SnCl_4^{2-} + Hg^{2+} + 2Cl^- \rightleftharpoons SnCl_6^{2-} + Hg^0(aq) \tag{6.2}$$

$$Hg^0(aq) \rightleftharpoons Hg^0(g) \tag{6.3}$$

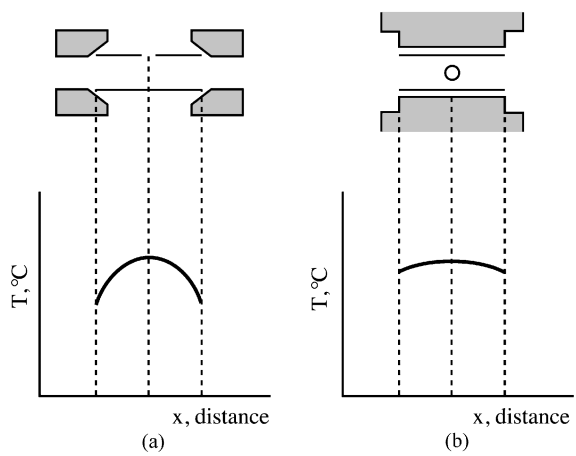


Figure 6.9 *Schematic representation of heating types in furnace atomization and typical temperature profiles along the cuvette: (a) longitudinally heated cuvette; (b) transversely heated cuvette*

Atomic aqueous mercury can then be drawn into gaseous phase by continuously supplied air that also carries the analyte into an absorption cell where a peak-shaped absorbance signal is formed. The gaseous product can then be safely trapped in a $KMnO_4$ solution. CVAAS system can be very easily manufactured in any laboratory. Analytical wavelength employed is 253.7 nm; quartz or silica windows are required for the absorption cell; the front faces of used HCLs may be an economical source of silica. The glass body can be manufactured in the shape of the source beam profile to attain better detection limits.[19] Experimental set-up for this technique is very similar to that used for hydride generation; this will be discussed in the next section. Amalgamation of Hg vapour with gold metal is used as a means of preconcentration prior to CVAAS analysis; there are now commercial systems using this principle.

There have been continuous attempts to speciate mercury as organic and inorganic, where $NaBH_4$ can reduce both the species but $SnCl_2$ can reduce only the latter.[20] Electrochemical reduction of organic compounds have also been used for speciation.[21] CVAAS system allows determination of Hg at levels below μg L^{-1}. Commercial CVAAS accessories using automation in a flow system are available both with and without amalgamation.

6.2.3.4 Hydride Generation Atomic Absorption Spectrometry

Another approach to higher sensitivity is in forming the hydride of the analyte, which is thermally less stable than its oxide that may commonly form in a flame. This method was introduced by Holak.[22] For some elements, the relatively stable oxides prevent the atomization of a good portion of analyte in flames, the hydrides of these elements can be formed in a reaction chamber situated before the atomizer. The hydride can then be efficiently and easily decomposed in a quartz tube heated by a flame or an electrical furnace. All the hydrides used with this system decompose at about 900 °C; this temperature is easily reached in a quartz atomizer mentioned above. It is possible to have detection limits at or below μg L^{-1} level. The technique is limited only to some elements; these are As (AsH_3), Se (H_2Se), Sb (SbH_3), Bi (BiH_3), Ge (GeH_4), Sn (SnH_4), Te (H_2Te) and Pb (PbH_4). Usually, $NaBH_4$ is added to analyte solution containing 1–6 M HCl causing the reactions below:

$$3BH_4^- + 3H^+ + 4H_3AsO_3 \rightarrow 3H_3BO_3 + 4AsH_3 + 3H_2O \quad (6.4)$$

$$BH_4^- + 3H_2O + H^+ \rightarrow H_3BO_3 + 4H_2 \quad (6.5)$$

The production of hydrogen radicals (H·) are also believed to be effective in formation of the atomic signal. An apparatus similar to that used for CVAAS is used where the hydride is formed in the chamber as described above and transported to the quartz tube furnace by the carrier gas, N_2 or Ar. The analyte plug passes through the quartz furnace, which functions both as the atomizer and the absorption cell; a peak-shaped signal is formed. The quartz furnace is T shaped; it receives the analyte plug in the centre. The quartz atomizer is heated either electrically or by a flame. As the gaseous products leave the quartz furnace from both ends, proper ventilation must be provided for safety reasons. Either the height or the area of the peak signal is used

for quantitation of the analyte. A simple and schematic representation of an HGAAS system is shown in Figure 6.10. Continuous flow systems are used, which allow automation of the technique; the analyte solution is injected into a stream of aqueous $NaBH_4$; depending on the amount of analyte, the signal may need to reach a plateau where it is then time-integrated. Since HGAAS technique provides the separation of the analyte as a gaseous product, some of the matrix interference problems are eliminated. However, the efficiency of hydride formation may be adversely affected by the presence of some transition metals such as Fe, Co and Ni that cause lower sensitivity.[23]

Experimental set-up for HGAAS can easily be manufactured in any laboratory; geometrical and experimental parameters should be optimized for such an apparatus. Automation, however, can be realized with commercial flow systems at the expense of spending larger amounts of chemicals as compared to the batch system. At the early stages of hydride generation techniques, the flames were also used as atomizers; since air–acetylene flame absorbs some in UV region where most analytes have their resonance lines, other flames, such as $Ar–H_2$ flame were used. Today, the most common atomizer for HGAAS is the quartz furnace, graphite furnace atomizers have also been used as atom cells.

6.2.3.5 Atom Traps for Flame Atomizers

Flame atomization, as compared to furnace atomization, is significantly more economical and less prone to interferences. Therefore, there has been a continuous effort to close the sensitivity gap between flame and furnace atomization. As a result

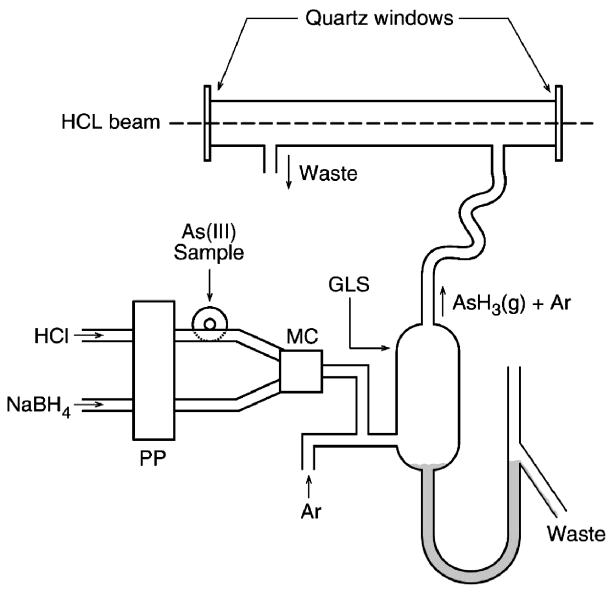


Figure 6.10 *HGAAS flow system for As determination; PP, peristaltic pump; MC, mixing chamber; GLS, gas–liquid separator; HCL, hollow cathode lamp*

of these efforts, there are now several devices termed as *atom traps* to be used with flame atomizers. The simplest atom trap is also called as *slotted quartz tube*; it is a quartz tube placed on the flame to increase the residence time of analyte atoms. The alignment is such that the source beam is fitted into this slotted tube in a way similar to CVAAS or HGAAS. A slot is made on one side that allows the entrance of the laminar flame partially into the tube. Usually, there is another slot at about 180° with respect to the first one. Sensitivity increase is 2–5 fold for some *volatile elements* such as Cd, Pb and Zn as compared to conventional flame atomization[24]; the signal is formed in a real-time mode by conventional sampling in the form of aspiration into the flame.

Another atom trap is a water-cooled silica tube that is placed on the laminar flame.[25] The sample solution is aspirated for about a minute and the analyte atoms are trapped on the cooled surface of the silica tube. The trapping tube is aligned in such a way that the source beam passes through just above the tube. After *in situ* preconcentration of analyte atoms, the cooling water is replaced by air; the analyte species collected on the tube form the atomic cloud again to cause a transient signal. Sensitivity enhancement can be as high as 50–100. Another approach has been suggested by using simply a slotted quartz tube; the flame conditions are optimized in such a way that the tube functions as a collector or an *atom trap*. After collection of atoms at optimized flame conditions, atomization is effected either by altering the flame conditions or simply by aspirating a small volume, *ca*. 50 μL of an organic solvent into the flame[26,27]; detection limits are at ng mL^{-1} level for volatile elements such as Pb and Cd. Initiating from this behaviour, an online hollow quartz tube has also been heated at selected collection and releasing temperatures to function as an *atom trap* that can preconcentrate analyte species after a hydride generation step for determination of Pb at ng L^{-1} level[28]; a flame-heated quartz tube was used as atomizer.

6.3 Interferences

Additive interferences may be corrected in the absence of analyte if an ideal blank can be prepared. *Multiplicative interference*, on the other hand, can only be observed and corrected in the presence of analyte. Interferences in AAS are best classified as *non-spectral* and *spectral interferences*. Non-spectral interferences are caused by several effects and can be named as, *physical (transport)*, *chemical* and *ionization interferences*. It should be noted that the presence of an *interferant* may be effective in more than one way. For example, presence of organic materials in an aqueous sample may alter the viscosity, and thus the suction rate of the sample in a flame-AAS determination, causing *physical interference*. In addition, the same interferant species may absorb light at the analytical wavelength causing *spectral interference*; they also may affect the formation and the population of free analyte atoms in the atomizer by solution or gas-phase reactions and thus cause *chemical (non-spectral) interference*. In general, interferences are more effective for sensitive atomizers; higher population and longer residence times for analyte species usually correspond to interference effects that are more difficult to deal with as compared to insensitive systems. Naturally, the interfering species are also in high concentration and they also may experience longer residence times in measurement zones.

6.3.1 Non-spectral Interferences

Difference in sample and standard solution viscosities will cause a *sample transport* or *physical* error, since the suction rate into the nebulizer will be lower for the more viscous solution. In addition, the solutions with lower viscosities will have better nebulization efficiencies as they can form a mist relatively more easily. Matrix simulation or standard addition techniques can be used to alleviate this problem. Presence of organic solvents will result in higher suction and nebulization rates as compared to the solutions where water is the only solvent, resulting in a positive error if aqueous standards were used. It should be noted that all the organic solvents involved in flame-AAS measurements are more volatile; therefore, mist formation is easier.

An important group of *non-spectral interferences* is *chemical* in nature. Any chemical reaction between the analyte and the interferants before, during and even after atomization may alter the free analyte population. This type of interference is *multiplicative* in the nature and thus the associated problems should be handled in the presence of the analyte. It should be remembered that a separation alone or a separation together with an analyte preconcentration step would remove these interferants. However, more chemicals and larger number of procedure steps are involved and these constitute the additional sources of errors. Both for the flame and electrothermal atomizers, absolute amount or concentration of the interferant has been found to be more effective than the interferant/analyte ratio. Therefore, dilution of the sample may eliminate some of the interferences. The limiting factor in this approach is the dynamic range of the system. Chemical interferences may be encountered under different names for flame and electrothermal atomizers for both practical and historical reasons.

Stable compound formation. This is typically observed in flame atomizers. Many oxygenated anions such as phosphate and sulfate depress the analyte signal in an air–acetylene flame, most notoriously for Ca and Mg. These anions form stable and involatile species with analyte, thereby lowering the free atom population in the measurement zone. There are several ways to deal with this type of interference:

1. *Using a hotter N_2O–acetylene flame*. Most compounds are decomposed in this flame and thus the analyte atoms are freed.
2. *Using a releasing agent*. Adding La or Sr chloride to the medium usually eliminates this interference. These added cations preferentially combine with the interfering anion and the analyte is freed. Alternatively, EDTA or similar chelating *protective agents* may be added; these reagents complex with analyte cation preventing the formation of the involatile species. Once in the flame, however, since these complexes are mostly organic in nature, they easily dissociate to give free analyte atoms.

Regarding the electrothermal atomizers, similar problems may arise. Many refractory elements, such as Ba, B, Mo, Ta, V and W, react with the graphite surface to form their stable carbide compounds. Employment of *pyrolytic graphite cuvettes* minimizes this problem. With the use of N_2 as a carrier gas during the ashing step,

formation of the stable nitrides is another problem; certain elements such as Al, Ba, Be, Ca, Cr, Ga, Ge, Hf, Li, Mg, Si, Sr, Ta, Ti, U, V and Zr are prone to this type of interference. Using Ar instead of N_2 as the carrier gas prevents this interference.

Volatile compound formation. This is mostly encountered during the ashing stage in an electrothermal atomizer. Many oxides and halides are formed and diffuse out of the cuvette without being atomized. The similar species may also form as the cuvette is heated up to the atomization temperature and if the heating rate is high enough, these volatile compounds decompose to give the free analyte atoms without having any chance to diffuse out of the atomizer, the measurement zone.

If these volatile compounds form during the ashing stage, the temperature programme must be altered to prevent the loss of analyte. For the volatile compounds formed just prior to atomization as the furnace is heated from ashing to the atomization cycle temperature, a high heating rate, using a platform or a probe, may alleviate the problem. Alternative solutions may be pursued during the ashing stage; a typical approach to remove chlorides is to add NH_4NO_3 to a sample that suffers volatile chloride formation problem[29]:

$$NaCl + NH_4NO_3 \rightarrow NaNO_3 + NH_4Cl \tag{6.6}$$

Decomposition temperature for NaCl is 1400 °C and thus this compound is stable during the most ashing temperatures. The products above, however, do not persist at temperatures higher than 400 °C; $NaNO_3$ decomposes at 380 °C and NH_4Cl is well known to become volatile easily, its boiling point is 330 °C.

Altering the course of chemical reactions in an atomizer is often termed as *matrix modification*; NH_4NO_3 is one of the first *matrix modifiers*.

In some cases for ETAAS, the analyte itself is too volatile and thus an efficient ashing is not possible. For example, Se is highly volatile especially when it is in organic form. In the presence of Ni, however, Se forms an intercalation compound with this element and is less volatile in this form; the sample can then be easily raised to temperatures as high as 1200 °C, allowing a successful ashing, as shown in Figure 6.11. Instead of Ni, Pd or a mixture of Pd and $MgNO_3$ has been suggested as an ideal matrix modifier for many volatile elements as well as for Se.[30] There is continuous research on the search for an ideal and universal matrix modifier for ETAAS analyses.

A careful examination of the temperature programme selected and a good understanding and appreciation of the chemical and physical events occurring in a cuvette are very useful and helpful regarding the precautions for interference elimination. On the other hand, chemical interferences are less common in flame atomizers since all the constituents are rather diluted; this is ironically due to the low nebulization efficiency in flame atomizers, which is frequently mentioned as a disadvantage regarding sensitivity.

Another well-known type of non-spectral interference is *ionization interference*. In hot flames, such as the N_2O–acetylene, alkali or earth alkaline elements ionize in a significant fraction:

$$Na(g) + Heat \rightleftharpoons Na^+(g) + e^- \tag{6.7}$$

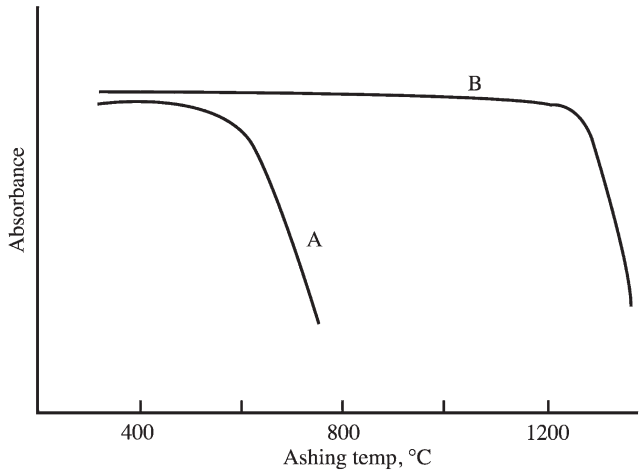


Figure 6.11 *Effect of Ni on volatilization of Se in ETAAS, shown by ashing plots. A: 1.0 ng Se(IV) in aqueous solution; B: 1.0 ng Se (IV) and 5000 ng Ni as* $NiNO_3$

This causes lowered sensitivity and a non-linear response in calibration plots. Another element with a lower ionization potential than that of the analyte may be added to the solution; for example, K may be used as an *ionization buffer* for Na:

$$K(g) + \text{Heat} \rightleftharpoons K^+(g) + e^- \tag{6.8}$$

Ionization buffer is added in a sufficiently large concentration, such as 100 mg L^{-1}. The electrons produced in the reaction (6.8) suppress the ionization of analyte in the Equation (6.7) according to the Le Chatelier's principle.

6.3.2 Spectral Interferences

Any kind of radiation that falls into the monochromator bandwidth, causing an emission or absorption signal at the detector, may be termed as a *spectral interference.* Naturally, stray light reaching the detector, which is the extraneous radiation that falls outside the intended monochromator bandwidth, is also a source of error; however, this phenomenon will not be discussed here since it is common to all spectrometric techniques.

A very simple kind of spectral interference is *emission from atomizer*, which may saturate the detector in some cases. Precautions are taken in particular with the graphite atomizers, as these will have a high blackbody emission from the furnace wall, probe or platform. For the ideal optical designs, the entrance slit of the monochromator is conveniently shielded from this sort of emission. This problem is actually solved as the spectrometer is designed. However, the user should be reminded that excessive misalignments of furnace atomizers might create this type of complication, which shows itself by excessive noise on signal profiles.

The spectral interferences may be combined in two important classes; namely, *line* and *broadband interferences*. It should be remembered that the spectral slit size, typically 0.5–2.0 nm, is much larger than the linewidth of radiation sources, about 0.005 nm, which determines the resolving power. Therefore, any species absorbing within the spectral slit range will not cause any interference unless a direct overlap with the source emission profile takes place. Such a direct overlap is called *line interference* and the best solution is to employ another analytical wavelength where there is no such overlap. One of the well-known line interferences occur during the determination of Al at 308.215 nm, where the presence of vanadium, which absorbs at 308.211 nm, causes line interference by a direct overlap. The remedy is to use the line 309.271 nm for Al. Line interferences are relatively rare and can be detected by the presence of positive systematic errors on analyte signal.

The presence of some species, such as halides of alkali and earth alkaline metals, some oxides and other volatile molecules cause a *broadband absorption*. These molecular species, unlike the atomic species, have rather broad absorption bands; the fine structure due to vibrational states may not be resolved by the monochromator; therefore, the absorption profiles appear as a continuum in a small spectral interval where the atomic absorption is to be measured. Occasionally, this continuum may be structured. In addition, the presence of particles in atomizer cause *light scattering*; this is different than absorption, but its net effect is again in the form of a broadband. This type of interference may be called *broadband interference*; it is also frequently named as *background absorption*. Since the problem is distributed all over the spectral slit range, the analyte beam power is also reduced, resulting in a positive error. Several types of broadband absorption by matrix components are shown in Figure 6.12. Flat, sloped and structured background absorption profiles may be encountered where the structure may bed due to other element atoms as well as the molecular vibrational bands. It should be stressed that broadband absorption problem is much more serious for ETAAS as compared to flame atomizers, especially in the region of 180–350 nm.

A group of instrumental designs have been suggested and these are known as *background correction techniques* in AAS. In this text, it has been noted in several

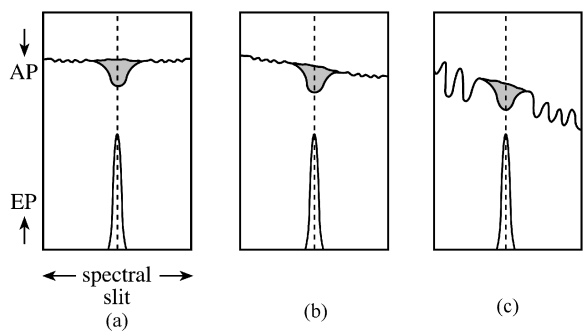


Figure 6.12 *Schematic illustration of several types of broadband absorption. EP: source emission profile; AP: absorption profile; shaded area represents analyte atomic absorption. (a) Flat background; (b) sloped background; (c) sloped and structured background*

occasions that a narrow source linewidth provides high wavelength selectivity in AAS that is not limited by the monochromator bandwidth. However, this spectral selectivity may be lost in some cases of background correction since these techniques require the consideration of the whole range covered by the spectral slit. This loss of spectral selectivity may cause other errors during the background correction. The performance and success of different background-correction approaches have been the subject of continuous study and discussion in literature and up to date no single background correction technique seems to perfectly solve all the broadband absorption problems in AAS. Background correction techniques most used in AAS are given below.

Two-line method. This is an approach used as the first solution to broadband absorption errors. Another wavelength in the vicinity of analyte line is used to measure the broadband absorption. It is assumed that the background absorption is *flat*, as shown in Figure 6.12(a). Analyte source radiation is absorbed both by the analyte and background species. A second measurement performed at a nearby wavelength provides the value of broadband absorption that is to be subtracted from the value found on analyte wavelength to give the net atomic absorption. The second line may be selected by using a source lamp of another element, which has a line very close to the analytical line; naturally this element should be absent in the sample. Alternatively, a non-absorbing nearby line of the analyte source lamp may be used. A third way is to use a nearby line from the inert gas in source lamp. In any case, two separate measurements have been necessary that rendered this approach rather impractical since the use of separate sample portions degrades precision and increases analysis time. Presently, this technique is not employed at all in the commercial AA spectrometers. Nevertheless, new instruments are being manufactured with the capability of simultaneous multi-wavelength measurements, using CCD or CID array detectors. Therefore, although now it seems to be obsolete, the two-line technique may find new applications with these novel instruments since only a single measurement would be sufficient for background correction. In addition, with the array detectors an accurate spectral characterization of background absorption and thus a proper selection of background wavelength can be made so that a good background correction can be realized. Recently, a portable AAS instrument using a W-coil atomizer and equipped with an array detector system has used the two-line technique for background correction. Recommended lines for this technique have been given in several sources.[31,32]

Continuum source technique. This approach was suggested by Koirtyohann and Pickett.[8] Two separate light beams are alternately sent through the measurement zone, from a line source of analyte and a continuum source such as a D_2 or an H_2 lamp. The principle of the technique is illustrated in Figure 6.13. Line source emission is absorbed by analyte atoms and background species, providing a signal which is the sum of atomic and broadband absorptions (AA + BA). When the mirror sector of the chopper is on the optical axis, continuum source radiation passes through the atomizer. Continuum radiation is absorbed by both the analyte atoms and the background constituents; however, the atomic absorption is a very small fraction of the total value and thus may be neglected; the signal represents broadband absorption (BA). The difference is taken and the net atomic absorption (AA) signal is

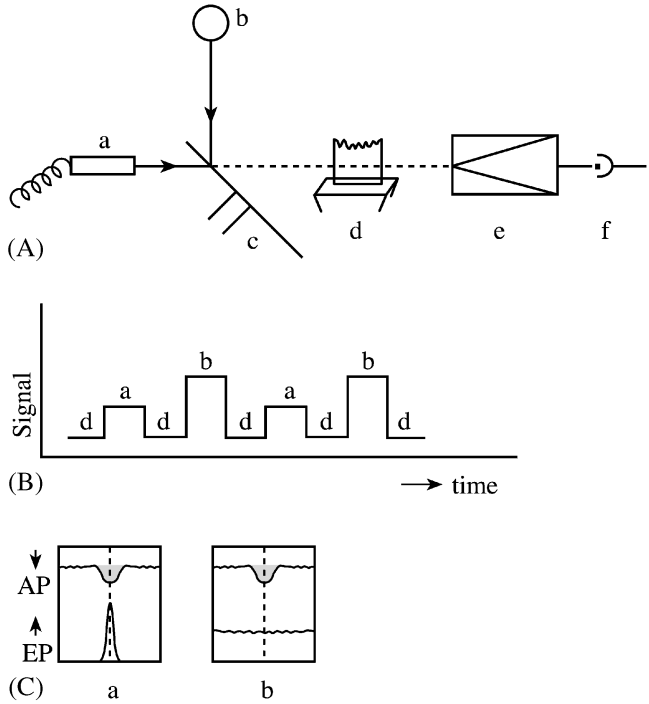


Figure 6.13 *Continuum source background correction technique. AA: Atomic absorption; BA: Broadband absorption. (A) Instrumental setup. a: Line source; b: continuum source; c: sector mirror (chopper); d: atomizer; e: monochromator; f: detector. (B) Modulated signals. a: Line source (AA + BA); b: continuum source (BA); d: Dark current. (C) Source emission (EP) and absorption (AP) profiles. a: Line source (AA + BA); b: continuum source (BA). Shaded area represents analyte atomic absorption*

obtained. Instead of mechanical chopping, a suitable pulsing regime for both the lamps may be used in most of the recently manufactured AA spectrometers employing continuum source technique for background correction.

D_2 arc or D_2 HCLs have been used in the UV region where the background absorption errors are most significant. Some instruments have an additional continuum source for VIS region, typically a W-lamp. Continuum source background correction technique has been commercialized and used widely. As mentioned before, when a continuum source is used for background correction, the wavelength selectivity of an atomic absorption measurement is practically lost. In this case, any interfering atom whose absorption profile falls in the range of monochromator bandwidth will cause an excessively and erroneously high background absorption signal and the *overcorrection of background absorption* will take place; both the sensitivity and accuracy will be degraded. Interference of Fe during Se determination has been a typical example for this type of error.[33] On the other hand, the volume in which the analyte atoms are observed should be exactly the same for both the line and the continuum sources; this requires a perfection in optical design; otherwise errors will

occur due to any misalignment. A modulation between two sources will create no time-based errors when a steady-state signal from a flame atomizer is used. However, a transient obtained from a graphite atomizer or HGAAS, CVAAS, *etc*., will need a fast monitoring especially on the ascending and descending sides of the peak-shaped signal. Earlier continuum source background correction designs used a modulation frequency of 30–70 Hz. Becoming aware of these time-based errors with peak-shaped signals, most novel AA instrument manufacturers now employ modulation frequencies as high as 200 Hz; therefore, the measurements of total and background absorption are made almost simultaneously.

Self Reversal (*Smith–Hieftje*) *Technique*. When an HCL is operated by sufficiently high currents, the emission profile is splitted into two peaks due to *self-absorption*. Ground-state atoms are relatively more abundant in the cooler section of the lamp discharge; therefore, suffering the broadening effects in lesser extent, they have a narrower bandwidth as compared to the excited-state atoms, which are relatively more abundant in the hotter sections. Consequently, when self-absorption takes place, the result is a self-reversal of the emission peak and a splitting at extreme current values. In this technique, the line source is pulse modulated by low and high currents. For the low-current mode, both atomic and broadband absorption (AA + BA) are measured.[10] The splitted emission profile is obtained with high current and is used for background absorption (BA) only, since the intensity is almost zero at the centre where the analytical line is located. The subtraction will give the net atomic absorption (AA), as illustrated in Figure 6.14.

This technique requires a single source and thus does not have the problems of source profile alignment as in the continuum source technique. However, specially designed HCLs are needed to standwith current modulation with acceptable life times. Since the splitting may not be perfect, background absorption contains some of the analyte atomic absorption signal, resulting in overcorrection. The use of this technique therefore causes non-linearity in the calibration plot, reduced sensitivity and even self-reversal at high absorbance values resulting in two concentration values for an absorbance reading.

Zeeman effect background correction techniques. Zeeman discovered in 1897 that spectral lines undergo splitting when atoms are placed in a magnetic field.[34] This is caused by the changes in atomic energy levels; a typical Zeeman splitting is shown in Figure 6.15 for Mg atoms. π and σ components are polarized in planes perpendicular to each other. With respect to the original resonance wavelength, π component has no shift, while the σ components are shifted about $\pm$ 0.01 nm around the centre. The total radiation power of π component is equal to the sum of σ components. When only 3 lines are formed, this splitting is termed as *normal Zeeman effect*, such as for Cd (228.8 nm), Hg (253.7 nm), Mg (285.2 nm), Pb (283.3 nm), Si (251.6 nm), Sn (224.6 nm), Sr (460.7 nm) and Zn (213.9 nm).

The Zeeman splitting may result in more than three lines; in these cases there are more than one π lines, one of which may or may not be coincident with the resonance wavelength. In these cases, the term *anomalous Zeeman effect* is used. Many important analytical lines, such as Se (196.0 nm), Al (308.2 and 396.2 nm), As (197.2 and 193.8 nm), Sb (217.6 and 231.1 nm), Cr (357.9 nm), Fe (248.3 nm), Ni (232.0 nm), Ag (328.1 nm), Co (240.7 nm), Cu (324.8 nm) and Mn (279.5 nm) show anomalous

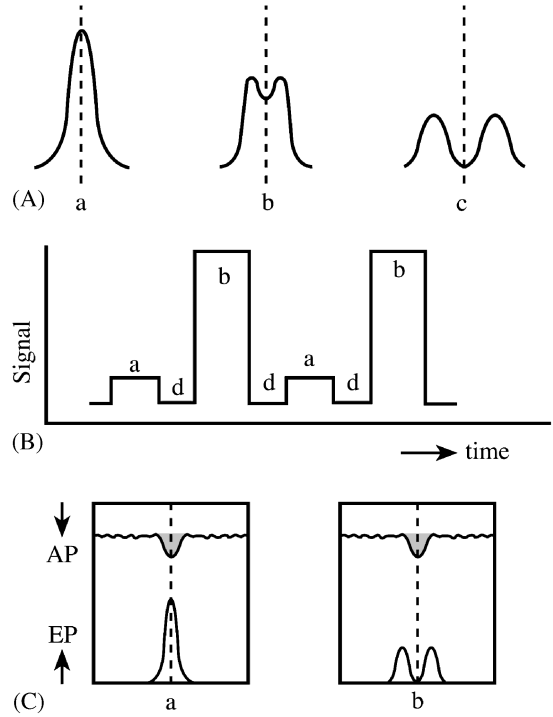


Figure 6.14 *Self-reversal background correction technique. (A) Splitting of an HCL emission profile by using a high current. a: Normal operation, low current; b: medium current, partial splitting; c: maximum current, maximum splitting. (B) Modulated signals. a: Low current, atomic and broadband absorption (AA + BA); d: Dark current; b: high current, broadband absorption (BA). (C) Source emission (EP) and sample absorption (AP) profiles. a: Low current (AA + BA); b: high current (BA). Shaded area represents analyte atomic absorption*

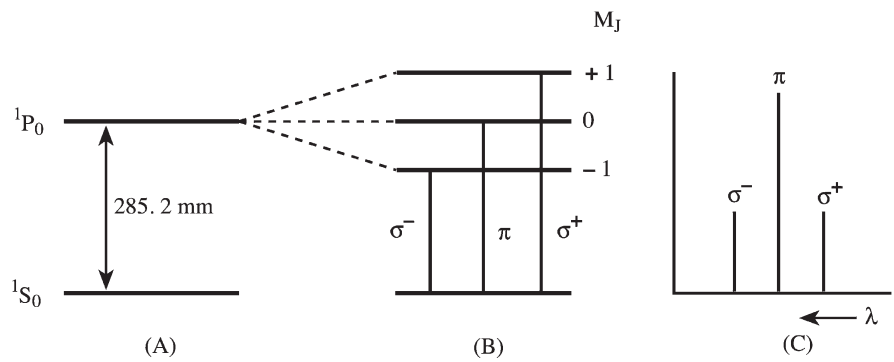


Figure 6.15 *The normal Zeeman effect for magnesium. (a) No magnetic field; (b) magnetic field, 10 kG, Zeeman splitting; (c) Zeeman effect causing a splitting of spectral lines*

Zeeman behaviour. The sorts of Zeeman splitting and splitted line patterns can be found in Welz' monograph.[35] In case of significant abundance of isotopes that have slightly shifted wavelengths, each isotope exhibits its own Zeeman-splitting pattern. Regarding the instrument designs, there are several types of Zeeman background correction. The magnetic field can be applied to the source of radiation; in this case, emission line profile undergoes Zeeman splitting; this configuration is called *direct Zeeman*. On the other hand, the magnet can be placed around the atomizer; absorption line profile splits; this configuration is called *inverse Zeeman*. During the Zeeman splitting, the polarization of the π and the σ components is termed according to the direction of observation, as shown in Figure 6.16 and described below.

Transverse Zeeman effect. In this case, the magnetic field is on the atomizer and applied perpendicular with respect to the optical axis of spectrometer. Therefore, the direction of observation is perpendicular to the magnetic field; π component is polarized in a direction parallel to the magnetic field; the σ components, on the other hand, are polarized in a plane perpendicular to the magnetic field.

Longitudinal Zeeman effect. The magnetic field situated around the atomizer is applied parallel to the optical axis. π components practically absent since its plane of polarization does not match with that of the π component of the emission from the source. σ components are displaced on the frequency axis and are circularly polarized.

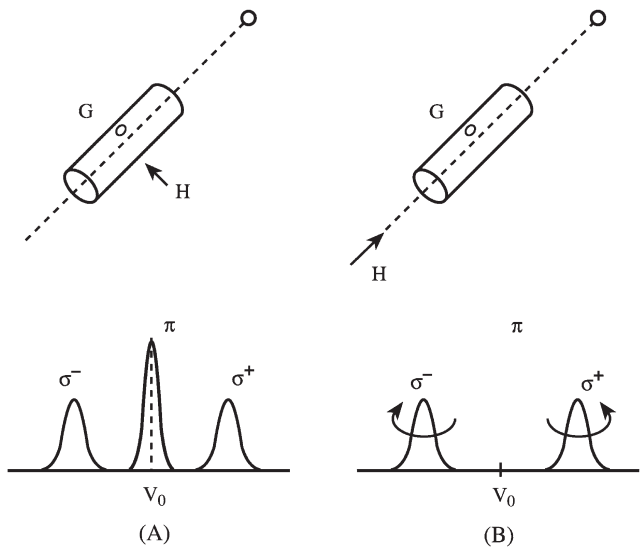


Figure 6.16 *Transverse and longitudinal Zeeman effect. H: Magnetic field direction; O: optical axis and direction of observation; G: graphite cuvette atomizer. (A) Transverse Zeeman effect*: *π component is polarized parallel, and σ components are polarized perpendicular to H. Radiation power is distributed as σ^- (25%), π (50%), σ^+ (25%). (B) Longitudinal Zeeman effect: π component is absent and σ components are circularly polarized. Radiation power is distributed as σ^- (50%), σ^+ (50%)*

After the review of common Zeeman configurations and definitions, the instrumental applications and their working principles can be discussed. Depending on the location of the magnet, application of magnetic field in a dc or ac and the modes of measurements, among the terms of direct, inverse, transverse and longitudinal, several kinds of Zeeman effect background correction designs are possible.[36] These are explained in the paragraphs below and in Figure 6.17.

Direct and transverse Zeeman AAS with constant magnetic field. The magnetic field is applied continuously (dc) and perpendicular to the optical axis and the magnet is on the emission source. A rotating polarizer is placed between the source and the atomizer, allowing π and σ components to be transmitted sequentially through the atom cloud. Only the emission profile is splitted. As the π component is used, both the broadband (BA) and atomic absorption (AA) are measured; σ component is used in another phase to measure BA only; the difference provides the net AA signal. This approach is usually called briefly as *direct Zeeman* in literature.

Inverse and transverse Zeeman AAS with continuous magnetic field. The magnetic field is applied continuously (dc) and perpendicular to the optical axis; the magnet is on the atomizer. Only the absorption profile is splitted. A rotating polarizer is situated

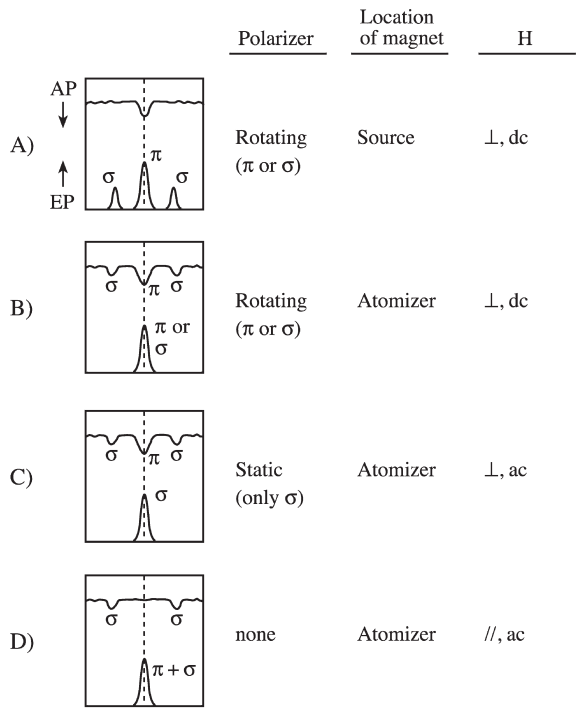


Figure 6.17 *Four important configurations for Zeeman background correction in AAS. EP: Source emission profile; AP: absorption profile. (A) Direct and transverse Zeeman AAS; (B) inverse and transverse Zeeman AAS-continuous magnetic field; (C) inverse and transverse Zeeman AAS-alternating magnetic field; (D) inverse and longitudinal Zeeman AAS-alternating magnetic field*

between the source and the atomizer, allowing alternatively the π and the σ components from the source, both on the same wavelength as the unsplitted analyte resonance line. The π component will be giving both the BA and AA signals; σ component will not be absorbed by the atoms whose absorption profiles are in π configuration, which is polarized in a different plane; therefore, only the BA signal will be registered. The difference is used to give the net A signal.

Inverse and transverse Zeeman AAS with alternating magnetic field. The magnet is on the atomizer and the magnetic field is applied alternatingly (ac), perpendicular to the optical axis; only the absorption profile is splitted. A static polarizer allows the transmission of only the σ component of the emission source; its wavelength is not shifted. Two phases are encountered with the magnetic field, *on* and *off*. When it is *off*, the absorption profile is not splitted, both π and σ components absorb at the central wavelength; (BA + AA) signals are registered. When it is *on*, the σ components from the source cannot be absorbed by the atoms that have the π configuration for the absorption profile; therefore, only BA is measured. The difference of these signals from the two phases provides the net AA signal.

Inverse and longitudinal Zeeman AAS with alternating magnetic field. The magnetic field is applied in an ac mode and parallel to the optical axis; the magnet is on the atomizer. In the absence of the magnetic field, (BA + AA) is measured. When the magnetic field is on, splitting takes place for the absorption profile; however, the π component for the absorption profile and the π component for the emission profile are on the different planes; no interaction occurs and therefore the π component for the analyte atoms is practically absent. The σ components of the absorption profile are circularly polarized and shifted in wavelength; therefore, there is no interaction between them and the σ components from the source emission. As a result, only BA is measured when the magnetic field is applied; this signal is subtracted from the (BA + AA) signal registered in the absence of the magnetic field, the net AA signal is thus obtained.

Each one of the four designs described above has their relative advantages and disadvantages. The direct Zeeman technique corrects the background signal using the σ components; although these are very close to the analytical wavelength, this correction is not exactly made at the resonance line where the atomic absorption signal is taken; in the case of structured background absorption, significant error may result.

6.4 Analysis of Solid Samples

In general, liquid samples are analyzed by both flame AAS and ETAAS. A dissolution step is necessary if the sample is a solid. Techniques and approaches to dissolve solids are given in Chapter 4. Dissolution process often requires the use of chemicals, contact of sample with several containers such as beakers and crucibles is unavoidable. All these operations increase the risk of analyte contamination and/or loss, resulting in deteriorated accuracy. Some laboratories have made systematic efforts to develop methods by which solids are analyzed without the need of a dissolution step. ETAAS as an atomizer is more suitable for direct sampling of solids. The main difficulty regarding solid sampling in ETAAS is that the samples and the standards should have exactly matched matrices to ensure accuracy. Although now

there is a large range and variety of certified reference materials (CRMs), in some cases calibration may be a problem.

Many liquid samples are not true solutions but actually contain solid materials suspended in aqueous media. Milk, fruit juices and some other beverages are in this class. Normally these samples require a dissolution step to be transformed to true solutions. However, *simple dilution and direct analysis* by flame or furnace atomizers is possible; in these cases the sample is treated as a true solution. Matched standards are required. Determination of Al by direct analysis of milk has been reported[37]; samples were only diluted (1+3) with 0.2% (V/V) HNO_3 prior to conventional procedure by ETAAS.

Another approach in direct solid analysis is *preparation of a slurry* or *a suspension of solid*, mostly in aqueous medium. The solid sample must be treated to have a well-defined particle size distribution. These samples can then be analyzed by conventional nebulization and a flame atomizer or deposited in a cuvette of an ETAAS system.[38,39]

Direct analysis of solids has the following advantages:

- Reduced sample preparation time.
- Reduced risk of analyte contamination and/or loss.
- Safer analysis environment as some of the corrosive reagents, such as acids, *etc*., are not used.
- Improved sensitivity in cases where the sample dilution is less than that in a dissolution procedure.

Among the disadvantages are the need for matrix-matched CRMs, the need for reproducing the particle size and errors associated with size distribution.

6.5 A General Evaluation and Capabilities of AAS Systems

There have been no exciting improvements in instrumental design characteristics of AA spectrometers in the last decade. Most of the instruments in the market are well designed, excellent spectrometers. Although very powerful techniques such as ICP-OES and ICP-MS are now available with multi-element capabilities and thus high sample throughput figures, AAS still survives in many laboratories, as it is comparatively economical and simple instrument.

Among the many designs offered in the market, the choices are to be made for the single- and double-beam spectrometers, Zeeman and D_2 background correction systems, instruments with different levels of automation and unattended operation, fairly different accessories for HGAAS and CVAAS, now mostly flow systems. The decision will depend on the specific needs and the workload of a laboratory. While a mostly academic research laboratory requires a flexible system, on the other extreme is a laboratory with a well-defined routine and heavy workload requiring a well-automated spectrometer to produce results accurate and fast. For the best economical decisions, one should always choose the least sensitive system if the problem could be solved this way. Therefore, if a simple flame atomizer is sufficient, the purchase of an ETAAS system is not needed until the new problems arise justifying its presence.

Feedback systems for most of the single-beam instruments are so perfect that the need for a double-beam design is not really justified; besides the latter has a poorer *S/N* value. Regarding which background system to prefer, although very often the Zeeman systems are presented as the best, the very same manufacturers having this claim continue to produce spectrometers with D_2 source background correction systems. The contemporary D_2 designs are now much improved with their high modulation frequencies; so that sudden changes in background signals can be successfully corrected. Instrument manufacturers have documentation illustrating the capabilities of background correction systems, the weakness and better performances of each background system for specific analysis problems. The customer with well-defined analytical problems should discuss these properties and decide accordingly.

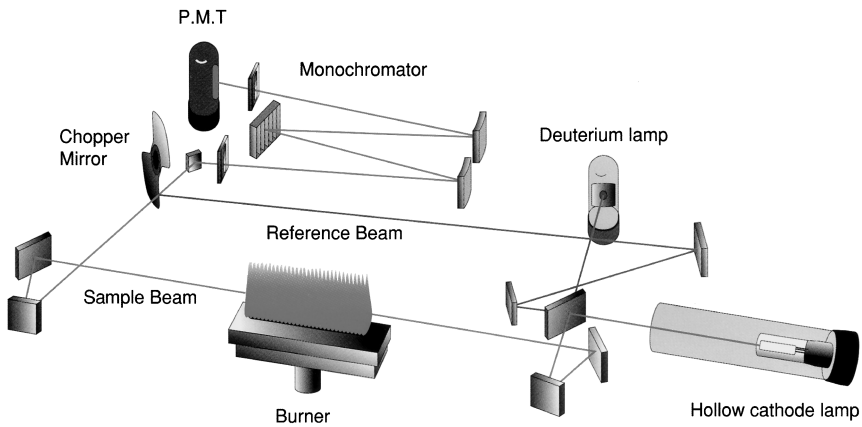


Figure 6.18 *Schematic illustration for AA-6200 spectrometer (Adapted with permission from Shimadzu Corporation, Japan)*

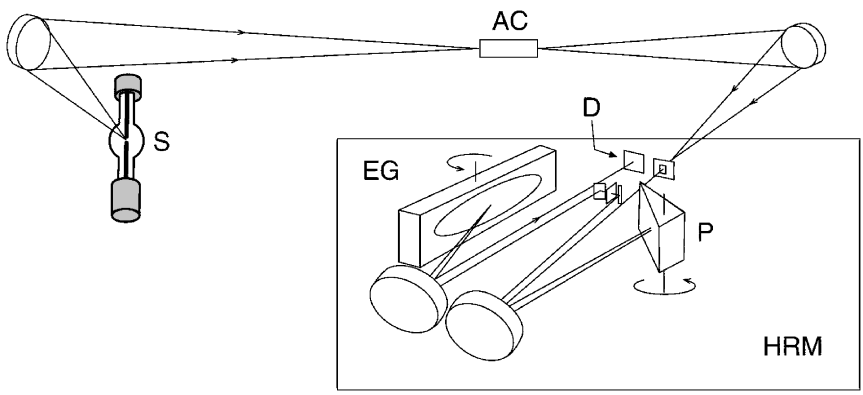


Figure 6.19 *Schematic illustration of contrAA continuum source, multi-element AAS system (Adapted by permission from U. Heitmann, ISAS, Department Berlin, Germany) S: Xe source; AC: atom cell, flame or furnace; EG: echelle grating; P: prism; D: detector, CCD; HRM: high-resolution monochromator*

Flow systems for flame, HGAAS and CVAAS are very commonly available. Home-made batch systems can be easily and economically prepared by the laboratories that may find these sufficient. Commercial flow systems provide higher sample throughput, well suited to heavy routine work.

A double beam AA spectrometer, Model AA-6200, manufactured by Shimadzu Corporation is shown in Figure 6.18. In such a typical double-beam instrument, D_2 background correction is sufficient to solve most of the background absorption

Table 6.1. *Detection limits using AAS techniques, µg/L[a] (Adapted from Reference 40)*

Analyte	*FAAS*	*HGAAS[b]*	*ETAAS*	*Analyte*	*FAAS*	*HGAAS[b]*	*ETAAS*
Ag	1.5		0.005	Na	0.3		0.005
Al	45		0.1	Nb	1500		
As	150	0.03	0.05	Nd	1500		
Au	9		0.15	Ni	6		0.07
B	1000		20	P	75000		130
Ba	15		0.35	Pb	15		0.05
Be	1.5		0.008	Pd	30		0.09
Bi	30	0.03	0.05	Pr	7500		
Ca	1.5		0.01	Pt	60		2.0
Cd	0.8		0.002	Rb	3		0.03
Co	9		0.15	Re	750		
Cr	3		0.004	Rh	6		
Cs	15			Ru	100		1.0
Cu	1.5		0.014	Sb	45	0.15	0.05
Dy	50			Sc	30		
Er	60			Se	100	0.03	0.05
Eu	30			Si	90		1.0
Fe	5		0.06	Sm	3000		
Ga	75			Sn	150		0.1
Gd	1800			Sr	3		0.025
Ge	300			Ta	1500		
Hf	300			Tb	900		
Hg	300	0.009[c]	0.6	Te	30	0.03	0.1
Ho	60			Ti	75		0.35
In	30			Tl	15		0.1
Ir	900		3.0	Tm	15		
K	3		0.005	U	15000		
La	3000			V	60		0.1
Li	0.8		0.06	W	1500		
Lu	1000			Y	75		
Mg	0.15		0.004	Yb	8		
Mn	1.5		0.005	Zn	1.5		0.02
Mo	45		0.03	Zr	450		

[a]All the values were found by using standard aqueous solutions and are based on 3s (98% confidence level). System 2 electrodeless discharge lamps were used whenever available with a Model AAnalyst 800 spectrometer. ETAAS detection limits were found by using 50 µL sample volumes, an integrated platform and full STPF conditions using AAnalyst 800 spectrometer.

[b]HGAAS detection limits were determined using MHS-15 Mercury/Hydride system.

[c]Hg detection limits by CVAAS technique were found by using a FIAS-100 or FIAS-400 flow injection system with amalgamation accessory.

problems. The flame atomizer can be replaced by a heated quartz atomizer for HGAAS or cold absorption tube for CVAAS. As almost all other AAS instruments in the market, Shimadzu AA-6200 is controlled and operated by a personal computer; a few adjustments can be done manually.

Recently, a continuum source AAS instrument has been manufactured by Analytik Jena A. G. The system employs a single continuum Xe source to cover a wavelength range of 189–900 nm, an Echelle monochromator and a CCD array detector system. This instrument is named as *contrAA* and is capable of multi-element determination in a sequential mode. A schematic illustration of contrAA is given in Figure 6.19.

A list of detection limits is given in Table 6.1; the values are provided by Perkin Elmer Instruments.[40] The values given in Table 6.1 are not necessarily comprehensive. Some of these elements can be determined by HGAAS technique although the table contains no such data; among these are Ge, Pb and Sn. On the other hand, although detection limits are given for some elements, their determination by AAS is not a part of common practice. Some of the rare earth elements are in this group, such as Gd, Lu, Nd, Pr, Sm and Tb. ICP-OES and ICP-MS techniques offer better detection limits for most of these elements. Table 6.1, however, contains the data available from the laboratory of a well-known manufacturer, and is very useful to have a general idea about the capabilities of AAS. Detection limits are available from literature and also from other manufacturers; the figures for an element may vary as much as 10-fold depending on the source. The user should remember that most of the commercial figures are for an easy water background. The detection limit for real samples and complex matrices may be about 10 times higher. It should also be remembered that LOQ values, determining the lower limit of actual applications, are about 5–10 times higher than detection limits.

References

1. A. Walsh, *Spectrochim. Acta*, 1955, **7**, 108.
2. C.Th.J. Alkemade and J.M.W. Milatz, *Appl. Sci. Res. Sect. B*, 1955, **4**, 289.
3. C.Th.J. Alkemade and J.M.W. Milatz, *J. Opt. Soc. Am*., 1955, **45**, 583.
4. B.V. L'vov, *Spectrochim. Acta*, 1961, **17**, 761.
5. A.S. King, *Astrophy*., 1908, **28**, 300.
6. H. Massmann, *Spectrochim. Acta*, 1968, **23B**, 215.
7. R. Woodriff and G. Ramelow, *Spectrochim. Acta*, 1968, **23B**, 665.
8. S.R. Koirtyohann, *Anal. Chem*., 1965, **37**, 601.
9. M. Prugger and R. Torge, *Ger*., 1969, **1964**, 469.
10. S.B. Smith, Jr. and G.M. Hieftje, *Appl. Spectrosc*., 1983, **37**, 419.
11. J.M. Harnly and T.C. O'Haver, *Anal. Chem*., 1981, **53**, 1291.
12. A.T. Zander, T.C. O'Haver and P.N. Keliher, *Anal. Chem*., 1976, **48**, 1166.
13. T.C. O'Haver, J.M. Harnly and A.T. Zander, *Anal. Chem*., 1978, **50**, 1218.
14. C.M.M. Smith and J.M. Harnly, *J. Anal. At. Spectrom*., 1995, **10**, 87.
15. D.J. Halls, *Anal. Chim. Acta*, 1977, **88**, 69.
16. D.J. Halls, *Spectrochim. Acta*, 1977, **32B**, 221.
17. W.R. Hatch and W.L. Ott, *Anal. Chem*., 1964, **40**, 2085.
18. T.R. Gilbert and D.N. Hume, *Anal. Chim. Acta*, 1973, **65**, 561.

19. G. Tuncel and O.Y. Ataman, *At. Absorpt. Newslett*., 1980, **1**, 126.
20. C.E. Oda and J.D. Ingle, Jr., *Anal. Chem*., 1981, **53**, 2305.
21. Ç. Ergüçyener, S. Aygün, O.Y. Ataman and A. Temizer, *J. Anal. At. Spectrom*., 1988, **3**, 177.
22. W. Holak, *Anal. Chem.*, 1969, **41**, 1712.
23. K.S. Subramanian and V.S. Sastri, *Talanta*, 1980, **27**, 469.
24. R.J. Wattling, *Anal. Chim. Acta*, 1977, **94**, 181.
25. C. Lau, A. Held and R. Stephens, *Can. J. Spectrosc*., 1976, **21**, 100.
26. N. Ertaş, D. Korkmaz, S. Kumser and O.Y. Ataman, *J. Anal. At. Spectrom*., **17**, 2002, 1415.
27. D. Korkmaz, S. Kumser, N. Ertaş, M. Mahmut and O.Y. Ataman, *J. Anal. At. Spectrom*., 2002, **17**, 1610.
28. D.K. Korkmaz, N. Ertaş and O.Y. Ataman, *Spectrochim. Acta Part B*, 2002, **57**, 571.
29. R.D. Ediger, G.E. Peterson and J.D. Kerber, *At. Absorpt. Newslett*., 1974, **13**, 61.
30. D.C. Manning and W. Slavin, *Appl. Spectrosc*., 1983, **37**, 1.
31. W. Slavin and G.R. Carnrick, *CRC Crit. Rev. Anal. Chem*., 1988, **19**, 95.
32. J. Sneddon, *Spectroscopy*, 1987, **2**, 38.
33. K. Saeed and Y. Thomassen, *Anal. Chim. Acta*, 1979, **100**, 285.
34. P. Zeeman, *Phil. Mag*., 1897, **2**, 226.
35. B. Welz and M. Sperling, *Atomic Absorption Spectrometry*, 3rd edn, Wiley-VCH, New York, 1999.
36. G. Rossi, in *Applications of Zeeman Graphite Furnace Atomic Absorption Spectrometry in the Chemical Laboratory and in Toxicology*, C. Minoia and S. Caroli (eds), Pergamon, Oxford, 1992, 3.
37. M.A.Z. Arruda, M.J. Quintela, M. Gallego and M. Valcárcel, *Analyst*, 1994, **119**, 1695.
38. N.J. Miller-Ihli, *J. Anal. At. Spectrom.*, 1994, **9**, 1129.
39. N.J. Miller-Ihli, *Fres. J. Anal. Chem*., 1990, **337**, 271.
40. Guide to Inorganic Analysis, PerkinElmer Life and Analytical Services, 2004, USA.

Further Reading

1. B.V. L'vov, *Atomic Absorption Spectrochemical Analysis*, (Translated by J.H. Dixon), American Elsevier Publishing Company, Inc., New York, 1970.
2. B. Welz and M. Sperling, *Atomic Absorption Spectrometry*, 3rd edn, Wiley-VCH, New York, 1999.
3. L.H.C. Lajunen, *Spectrochemical Analysis by Atomic Absorption and Emission*, The Royal Society of Chemistry, Cambridge, 1992.
4. J.R. Dean, *Atomic Absorption and Plasma Spectroscopy*, 2nd edn, Analytical Chemistry by Open Learning, Wiley, Chichester, 1997.

CHAPTER 7

Atomic Emission and Mass Spectrometry using Plasma Techniques

7.1 Introduction, History and Principles

Atomic emission spectrometry (AES) is one of the oldest analytical techniques. Since the emission signals in visible region are readily detectable by the human eye, the qualitative and quantitative use of them were discovered rather early when compared to other analytical techniques with signals not so readily detected by human senses, such as absorption. The dispersion by prism and diffraction gratings were discovered and applied by Newton in 1740 and Rittenhouse in 1786, respectively. Therefore, the necessary optical instrumentation for pioneering spectrometric studies was already available in the beginning of the 19th century. In 1802, the continuous emission spectrum of Sun was studied by Wollaston; the dark lines due to atomic absorption by the elements in Sun's atmosphere were later studied by Fraunhofer and Brewster. The relation between the spectra and the electrode material for a spark was discovered by Wheatstone in 1834. In 1859, Kirchhoff and Bunsen constructed the first flame spectroscope; they also explained the dark lines in Sun's spectrum as a result of absorption by atoms. In AES, the part of the system providing the energy for atomization, excitation and frequently ionization of analyte species is called the *source*.

The analytical use of electrical arcs and sparks started in the middle of the 19th century. In 1861, Kirchhoff was able to generate *sparks* across a gap using a combination of resistance and induction coils. In 1872, Lockyer investigated these sparks by using a spectroscope that consisted of a prism monochromator with visual examination of optical signals on the focal plane. In 1874, Lockyer was able to observe that the qualitative aspects of the sample depended on position, wavelength of the lines and the quantity of the species depended on the brightness of the signal; he therefore established the principles of modern AES. The first use of graphite electrodes was realised by Hartley in 1882. On the other hand, in 1870 the first *DC arc* was obtained by using a number of batteries. The DC arc provided a temperature of 6000–8000 K that was sufficient to melt and vaporize metal electrodes.

In 1920s, *arc and spark emission sources* were available for qualitative and quantitative AES. With continuous developments and evolution, arc and spark techniques survived until today, since they are still the best choices for rapidly producing data for metal industries using solid samples directly. Another major breakthrough has been the use of *inductively coupled plasma* (ICP) sources for AES. Two research groups led in the United Kingdom by Greenfield[1] and in the United States by Fassel[2] used ICP source for AES. Since ICP source provided emission signals for both atoms and ions, most scientists prefer to use the term *optical emission spectrometry* (OES) instead of AES; this abbreviation will also be used in this text. ICP-OES now has been widely accepted and became a very powerful tool for quantitative analysis. In the last 20 years, an important development in this field has been the use of mass spectrometric detection for the ions formed in an ICP[3]; determination at nanograms per litre levels for most of the elements is now possible by the use of this technique called as *inductively coupled plasma–mass spectrometry* (ICP-MS).

In order for an atomic emission signal to be generated, upper atomic energy levels should be populated. According to the Boltzmann distribution, this population is enhanced with low-excitation energies and in high temperatures. Plasma sources, providing temperatures as high as 10 000 K has made AES a highly sensitive technique. When the conditions are met, all the elements present in a sample exhibit their both atomic and ionic emissions as line signals. Therefore, unlike ordinary AAS, the analytical signals for all the species present are formed and thus can be detected when proper instrumentation is available. Consequently, by the nature of the analytical signals formed in a source, AES is a *multi-element technique*. In addition, most of AES techniques, especially plasma oriented ones, have large dynamic ranges reaching to 5–6 orders of magnitude. Combined properties of multi-element detection, high sensitivity and large dynamic range have rendered modern AES techniques very powerful analytical tools in practice.

The principles of MS have been known since 1920s; its application to molecular analysis has been widely and very efficiently used. Application of MS to atomic clouds for trace element determination started with invention of the spark source by Dempster in 1934. *Spark source mass spectrometry* (SSMS) has been applied for mostly inorganic elemental analysis with detection limits in the range of 0.1–1.0 ppm for solids. A high-voltage RF spark of about 30 kV is used to form ions from solid sample in an evacuated chamber.

The technique has a relatively low precision as the RF spark cannot be formed reproducibly. The accuracy can be improved by the use of internal standards. The use of SSMS for elemental analysis has also been limited because of difficulties in reproducibly volatilizing inorganic matrices and the need to break the vacuum for every new sampling that necessitated lengthy evacuations periods. Especially after the advances in the use of Ar plasma as an ion source, SSMS has left its place to ICP-MS for elemental analyses.

In this chapter, flame, arc, spark and other sources will be briefly mentioned. More emphasis will be given to ICP-OES and ICP-MS. It should be remembered that although there are several approaches to be classified, the techniques that are most directly and commonly applied for food and diet analysis are ICP-OES and

ICP-MS. Rather rare but possible other choices are *flame photometry* and *flame atomic emission spectrometry* where an AA instrument is used in emission mode.

7.2 Optical Emission Spectrometry

In this part the classical sources for AES will be covered first to be followed by the most used argon plasma sources.

7.2.1 Optical Emission Spectrometry with Classical Sources

Among the classical sources, flame, arc and spark have been used as analytical tools for almost last 100 years. Even after the introduction of plasma sources, the classical emission methods remained in use. On the other hand, flame photometry is still popular for its simplicity and low-cost instrumentation; where arc and spark techniques are also in use for their direct applicability to solids in metal industries.

Flame atomic emission systems are either low-cost flame photometers or commercial AAS instruments operated in emission mode. *Flame photometers* are simple instruments employing bandpass type filters for determination of groups IA and IIA elements. In practice, Na, K, Li, Ca and few other element determinations can be carried out by using flame photometers; this application is very common in clinical and many other laboratories. Since alkali (group IA) and most earth alkaline (group IIA) elements have rather long analytical wavelengths, corresponding to low-excitation energies, excited states are sufficiently populated even in an air–propane flame with an average temperature of 2200 K. Many laboratories equipped with a natural gas supply that is mostly consisting of methane, use air–natural gas flames for flame photometry.

On the other hand, it should be remembered that every AAS instrument could be operated in emission mode for AES. Since the flames employed are not nearly as hot as arc, spark and plasma sources, the background spectra are fairly simple; therefore, low-resolution monochromators can be employed. As a result, flame emission spectrometry rarely suffers line interferences even though low-resolution monochromators or filters are used as wavelength selection devices. In order to correct any flat or sloped background emission, measurements at either one or both sides of the analytical line can be taken and utilized. In many cases, signal to background ratio is sufficiently high so that a background correction may not be required at all.

Small variations in flame temperature causes significant changes in excited state populations, resulting in random errors in atomic emission signals. According to the Boltzmann equation, since the ground state population is much higher as compared to the excited state population, the atomic absorption signal is not significantly affected by temperature variations as much as the emission signals. However, the former statement is correct only if the total free analyte concentration remains unchanged upon temperature variations. In other words, at the temperature applied, atomization must be complete. Any flame temperature change affecting the total free atomic analyte concentration will induce errors and affect the signals also in AAS. The internal standard technique may be employed to minimize random errors caused by temperature variations in AES.

In contrast to the wide acceptance of flame as an atomic emission source, the use of furnace atomizers for AES has remained only as a research topic.[4] In theory, graphite furnace with a polychromator can combine the advantages of sampling at microliter levels and high sensitivity of electrothermal atomization with the multi-element detection capability of AES. In some of these studies commercial graphite furnaces coupled to Echelle type polychromators were used for multi-element analysis.[5]

Although the electrical arc and spark were put into analytical use at about the same time as flame photometry, the former approach had some different properties and advantages. In flame photometry, the source temperature is fairly low; only alkali and earth alkaline elements with low excitation energies can be determined; most commonly used lines are those due to the transition between the first excited state and the ground state. However, these lines are very intense and are fairly broadened in an arc or spark; dynamic range is limited because of self-reversal of signals at high analyte concentrations. On the other hand, in the temperatures as high as 6000 K, the upper states are also fairly populated and thus the transitions between the excited states can be used when electrical arc or spark is used as a source. These lines are less intense, self-absorptive and thus self-reversal is not observed; a long and useful dynamic range is obtained. The other different features of electrical arcs are the wider applicability on the periodic table and the high sensitivity obtained as compared to the flame sources.

Since the information for many elements are available in the form of emission signals, electrical arc and spark sources provide multi-element detectability that is inherent in any emission technique. The early instruments used prism and later grating polychromators with photographic detection at the focal plane by using a photosensitive plate or film. Usually, a photographic image of Fe was used as the wavelength standard. The polychromator must have the high resolution required to isolate the individual emission signal, since in these hot sources many states of concomitant species are also excited, resulting in a complex spectrum. After photoelectric detection was discovered, one or several slits were used on the focal plane of a polychromator; each one was coupled with a photomultiplier tube (PMT). The selection of the analytical lines depends on the chemical matrix; therefore, a certain set of analyte lines are used for a defined set of chemical analysis in a defined matrix. In other words, while all the spectral information would be available on a photofilm, only the selected lines are observed in a photoelectric detection. In this regard, the flexibility of a multi-element photoelectric detection is limited and a change in the nature of chemical analysis necessitates the use of some other lines; the position of the exit slits must be changed. The slits are set and can be changed by the manufacturer only. It has been possible to detect at most about 60 elements by such multi-element instruments. Nowadays, with advances in use of array detectors measurement at all wavelengths is possible again; in a way, this is similar to days of photofilms and photoplates but with powerful data acquisition systems provided by computers.

The most used electrical discharges are the *DC arc* and the *AC spark*. The DC arc is formed by a relatively high current of 5–30 A and a low voltage of 10–30 V. The arc is formed in the gap between the electrodes; air is used as the medium gas. One of the electrodes is pure carbon (counter electrode) and the other one (sample electrode) contains the sample either in the form of a pure metal or an alloy. The sample

can be used directly as one of the electrodes. The samples of different volatility pose a problem; pre-ashing of the sample may be needed. The resulting ash in the form of oxides can be mixed with large excess of graphite or silica, and placed in the cup-like lower (sample) electrode. Despite its high sensitivity, DC arc source suffers from fairly low reproducibility.

AC spark discharges with duration of 10–100 μs provide better reproducibility as compared to the DC arc, since the average of many discharges is taken. An alternating current with a 10–50 V potential is employed. This system, however, is less sensitive than DC arc instruments. The contemporary commercial emission spectrometers allow the use of many different states ranging from pure DC arc to complete AC spark; considering the sample matrix and the analytical problem, an optimization of best sensitivity and reproducibility is thus obtained. These systems based on arc and spark sources are basically for solid samples and obviously are still very useful tools in metal industries. The use of liquid samples has also been possible by introducing the sample solution into the electrical discharge in a continuous and reproducible manner. A preliminary but semi-quantitative check by an emission spectrometer is extremely useful even for today. Therefore, many laboratories, especially in mining industries have used emission spectrometers for this purpose. On the basis of the preliminary results obtained for many elements, further strategies for detailed quantitative analyses can be planned using other more precise and accurate techniques; saving a great deal of time and money.

7.2.2 Optical Emission Spectrometry with Plasma Sources

Since the higher temperatures are needed for better population of the upper electronic states, there has been a continuous search to design hotter sources. Plasma sources, especially using Ar and He, have provided the high temperature needed up to 10 000 K and resulted in higher sensitivity in AES. There are fundamental differences between the combustion flames such as air–C_2H_2 and plasma. Flame requires an oxidant and a fuel; its medium contains combustion products. Plasma on the other hand does not involve combustion or any other chemical process. *Plasma* can be defined as a high-energy medium where an appreciable fraction of the atoms are ionized. In most cases, more than 1% of the total atom population are ionized; therefore, the contents of plasma are atoms, ions, electrons and some neutral molecules. Plasma conducts electricity and is affected by magnetic fields; it provides a medium that is energetic enough to atomize and even ionize refractory carbides and oxides that commonly yield very low free atom populations in combustion flames. Although many kinds of plasma with different gas environments have been reported, Ar ICP is the most popular source today. The other used plasma sources are *direct current plasma* (DCP), *microwave induced plasma* (MIP) and *glow discharge* (GD).

Highly energetic and very popular ICP source is a result of energy transfer from a radio frequency (RF) magnetic field to an inert gas, mostly Ar. The gas is forced to flow in a direction perpendicular to the magnetic field applied; a spark is created usually by a Tesla coil to initiate the plasma. Only few Ar atoms are ionized in the beginning stages; energetic collisions of the species, Ar, Ar^+ and electrons, cause other ionizations resulting in the Ar plasma. By careful manipulation of the gas

flows, the plasma is sustained without touching the *torch* that is used to direct the gases and sample. Temperatures as high as 10 000 K can be reached; however, the most useful analytical regions of plasma are about 6000 K. A schematic representation of an Ar ICP is shown in Figure 7.1.

The fundamental analytical characteristics of an Ar ICP are as follows:

- The hot source (~6000 K) can break almost all the molecular bonds; refractory carbides and oxides are atomized. Energy is sufficient even for appreciable ionization of the analyte atoms.
- Observation of analytical species is performed at a certain height above the load coil where the background emission is low, so that *S*/*N* is favourable. The structure of plasma allows almost no self-absorption. Therefore, linear dynamic range is wide, as much as 10^4.

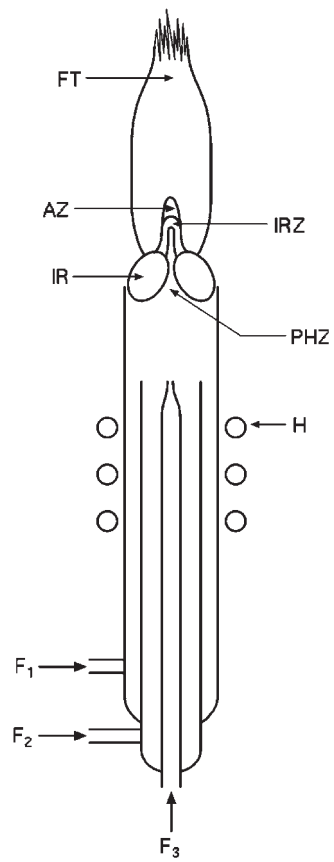


Figure 7.1 *Schematic representation of an Ar inductively coupled plasma. H, Cu load coil to induce magnetic field; F1, tangential plasma Ar flow, 7–15 L min⁻1; F2, auxiliary Ar flow, 1–3 L min⁻1; F3, sample or nebulizer flow (Ar + sample), ~1 L min⁻1 Ar and ~1 mL min⁻1 sample solution; IR, induction region; IRZ, initial radiation zone; PHZ, preheating zone; AZ, analytical zone; and FT, flame-like tail*

- Due to the high temperature, only few molecular species are present. There are no combustion products as in the flames. Therefore, the environment is *chemically inert.* Reduction of free atom population due to molecular reactions is minimized; chemical interferences are rare.
- High-temperature medium causes the population of many upper electronic states. Therefore, there are many possibilities for the choice of wavelength for an analyte. While this situation provides more analytical choices in the spectral domain, since the same conditions are also valid for all the other species, the resulting spectrum is rich in potentially interfering lines. An optical system with high resolution is thus required; consequently, the cost of instrumentation is higher than that for AAS systems that can be operated with rather low cost monochromators.
- As any other AES technique, ICP-OES is capable of multi-element determination. This has been done in a *sequential mode* using monochromators or in a *simultaneous mode* using polychromators. Since array detectors are widely available today, most instruments manufactured have simultaneous design.
- As well as the high temperature medium, the rather long residence times, such as 2 ms for analyte species in plasma causes atomization, ionization and excitation of both atoms and ions. Atomic lines and singly charged ionic lines are denoted as M(I) and M(II), respectively.
- Plasma is very rich in electrons; therefore, few ionization interferences are encountered.
- There are no electrodes; an inert gas is used. Its operation is rather simple and safe.

All of these properties have caused Ar ICP source to become a very powerful and common analytical tool. Detection limits are similar or better as compared to flame AAS. One of the most important advantages of ICP-OES is that even the refractory elements such as B, Al, Si, W, Zr, Ta and rare earth elements can be determined; these analytes pose difficulties in combustion flames. Ar and atmospheric gases are present in large concentrations in plasma, so that the determination of Ar, H, O, C and N is not practicable. Halogens, F, Cl, Br, I and noble gases such as He, Ne, Kr, Xe, Rn cannot be excited and thus cannot be determined by ICP-OES technique. Radioactive elements with short half-lives are also not on the list of analytes.

7.2.2.1 Power Supplies for RF Generation

Ar ICP receives its energy from the magnetic field imposed; this energy transfer, or the *coupling process*, from magnetic to electrical field provides the energy to create and sustain the plasma. RF generators operating with a power of 500–2500 W are used for this purpose. RF employed is between 27 and 60 MHz. Initially, most ICP instruments used 27.12 MHz; presently, 40.68 MHz is more popular, since it is easier to sustain the Ar plasma under variable conditions at this frequency.

Crystal-controlled generators use a piezoelectric quartz crystal to produce RF signal, which is then amplified and applied to a Cu load coil around the plasma torch. The other and presently more commonly employed system is the *free-running generator*,

which is capable of automatically altering its oscillation frequency upon the changes in plasma characteristics. Therefore, when the samples with different chemical compositions are introduced into the plasma, varying in salt or organic content, for instance, a new frequency required to sustain the plasma is chosen; the extinguishing of plasma thus is prevented. Free-running RF generators are smaller and more inexpensive when compared to the crystal-controlled RF generators.

7.2.2.2 Sample Introduction Systems

Modern ICP-OES instruments are very capable multi-element systems using powerful optical, electronic and computer technology. However, the sample introduction into plasma is often referred as the weakest ring of the chain or the *Achille's heel* for the system as suggested by Browner and Boom.[6]

Sampling Solutions: Mostly liquid samples are handled in ICP-OES and usually these are aqueous. The problem is the transportation of sufficient amount of analyte into the plasma without altering plasma characteristics to obtain a reproducible and accurate analytical signal. The sample transport system consists of a *nebulizer* to form small droplets, which is followed by a *spray chamber* to eliminate the large droplets and finally the *sample flow of Ar* to introduce these selected droplets with a size of less than 5 μm into the center of the plasma. Typical sample gas flow is 1.0 L min^{-1} Ar and commonly the rate of liquid sample transport into the system is 1.0 mL min^{-1}. The overall efficiency of the system for transportation of analyte species from the sample solution into the plasma is 1–10%; this figure can somewhat be improved by using better means of nebulization. An overall liquid sampling system with a nebulizer is shown in Figure 7.2.

Nebulizers are classified and used in the following groups:

Pneumatic nebulizer. The gas flow in a chamber creates a vacuum into which the sample solution can be sucked by the process known as the *Venturi effect.* The principle of pneumatic nebulization is the same as that is used for flame AAS. The important difference between these systems are typical flow rates which are about 5.0 and 1.0 mL min^{-1} for AA flames and Ar ICP, respectively. This difference is caused by the fact that a rather high total gas flow is used in AA flame, such as 10–20 L min^{-1}, where Ar ICP sample flow is about only 1.0 L min^{-1}. The most common kinds of pneumatic nebulization systems are *concentric*, *cross-flow* and *V-groove nebulizers* as shown schematically in the Figure 7.3.

Ultrasonic nebulizer. The sample is directed onto a piezoelectric crystal plate vibrating at a frequency of 0.2–10 MHz. The nebulization takes place on the vibrating plate; the aerosol thus formed is directed through first a heated chamber, then a cooled one; desolvation is thus realized as the solvent volatilized in the heated chamber will be condensed in the following one. In addition to improved nebulization, the sample is directed into plasma without its solvent. Consequently, there is no cooling effect in plasma. Flow rate for the transport of fine, dry sample particles into plasma is lower as compared to pneumatic nebulizers. This results in longer analyte residence times in plasma up to 2–3 ms that is another cause of improvement in sensitivity. The main advantage of this nebulizer is about 10 times enhancement in sensitivity.

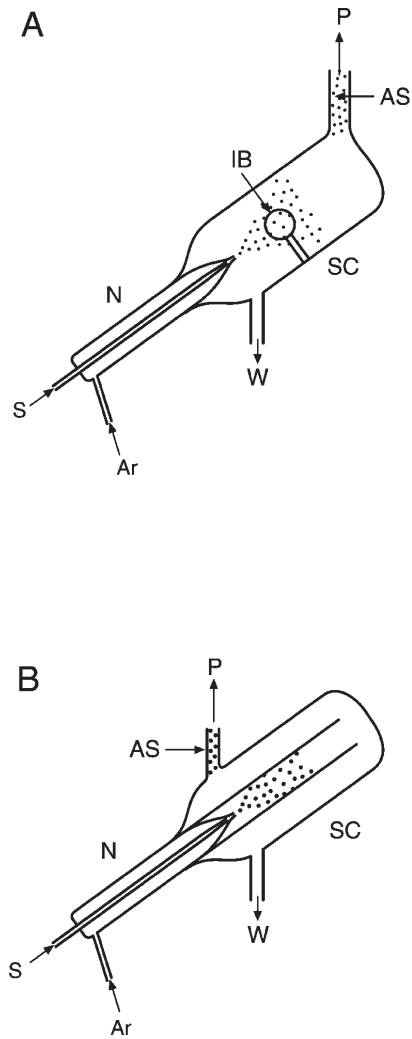


Figure 7.2 *Schematic sample introduction systems for ICP-OES. (A) impact-bead spray chamber, (B) double-pass type spray chamber. N, nebuliser; SC, spray chamber; P, plasma sample flow; Ar, Ar flow; IB, impact bead; S, sample flow; AS, aerosol sample (small droplets, $d < 5$ μm); and W, waste*

Difficulties and memory effects are experienced with samples of high solid content. Accumulation of non-volatile components on the vibrating crystal causes alteration of the vibrating frequency, that will in turn reduce the nebulization efficiency.

Grid nebulizer. Hildebrand grid nebulizer[7] uses two successive platinum grids through which the sample solution is forced to pass. The aerosol is produced after the first grid; the second grid that is about 2 mm away from the first one breaks up

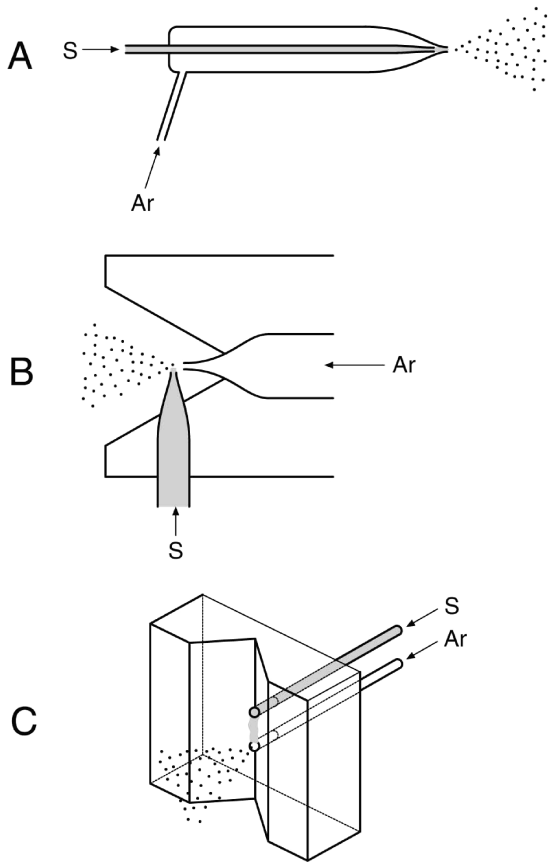


Figure 7.3 *Schematic representations of some pneumatic nebulizers. A, concentric nebulizer (low solid content); B, cross-flow nebulizer (high solid content); C, V-groove nebulizer (high solid content); S, sample flow (usually by a peristaltic pump); and Ar, Ar flow*

the aerosol into smaller particles. The sample mist is directed to plasma through a spray chamber. Schematic representation of a grid nebulizer is given in Figure 7.4. Extensive use of solutions containing aqua regia may harm the platinum grids.

In order to ensure a reproducible sample solution transport into a nebulizer, a peristaltic pump is almost always used. However, if there are significant differences in viscosities of sample and standard solutions, nebulization efficiencies will be different and thus the net analyte transport efficiency to plasma will show variations; internal standard technique can be used to correct the induced errors.

Vapour generation techniques. For sampling solutions, in addition to introducing solutions directly to nebulizers, *hydride generation* for As, Se, Sb, Sn, Bi, Te, Ge, In and Pb and *cold vapour formation* techniques for Hg and Cd are also frequently used in ICP-OES. The principles are the same as described in Chapter 6. Automated systems based on flow injection are often used for these vapour generation techniques.

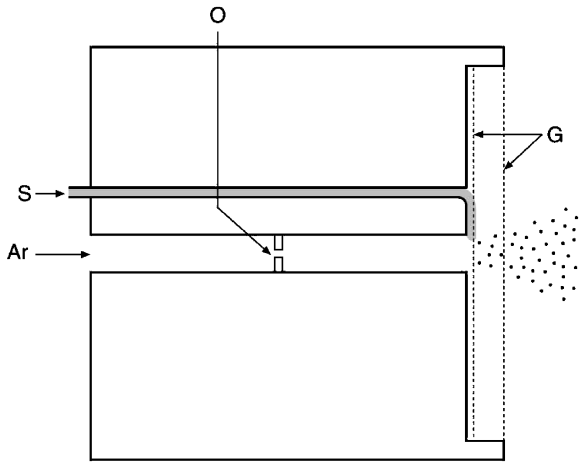


Figure 7.4 *Schematic representation of Hildebrand grid nebulizer. S, sample flow; Ar, Ar flow; O, orifice; and G, platinum grids (Adapted from Ref. 7 with permission from Society for Applied Spectroscopy, Frederick, MD, USA and Professor M. B. Denton)*

Sampling Solids: Analysis of solids has also been possible although it is not as easy and convenient as in the case of liquid samples. Numerous ways of solid sampling have been proposed, the most popular techniques are *electrothermal vaporization* (ETV) and *laser ablation* (LA).

In principle, ETV is a graphite vaporizer–atomizer; its essential function is vaporization rather than atomization when used with ICP-OES. The working principles of graphite atomizer are given in Chapter 6. When coupled to ICP-OES, however, the transport of vaporized sample into plasma becomes important; the locations of vaporization and measurement have thus been separated. All the advantages of ETA, such as ashing with a convenient temperature programming and using chemical modifiers are also valid for ETV. Both tube and rod types of atomizers have been used.

In contrast to nebulization of solutions, LA technique can produce high transport efficiencies, approaching to 100% in some cases, but only for a brief period of few seconds. The signal obtained is transient. This technique is based on vaporization of solid samples by using a powerful laser beam such as a Nd-YAG laser. Since the laser beam can be focused on areas as small as micrometer dimensions, LA technique can be used for localized analysis of heterogeneous materials. Depth profiling is also possible. A schematic representation for LA system is given in Figure 7.5.

7.2.2.3 Detection Systems and Measurement Modes in ICP-OES

Depending on the needs of analyst and the design characteristics of instruments, several modes of measurement have been possible in ICP-OES determinations. Although ICP-OES can be used as a single-element technique, its multi-element capability is always present and is often used. When a monochromator with a single exit slit and a single detector is employed, the dispersing device rotates to select the desired

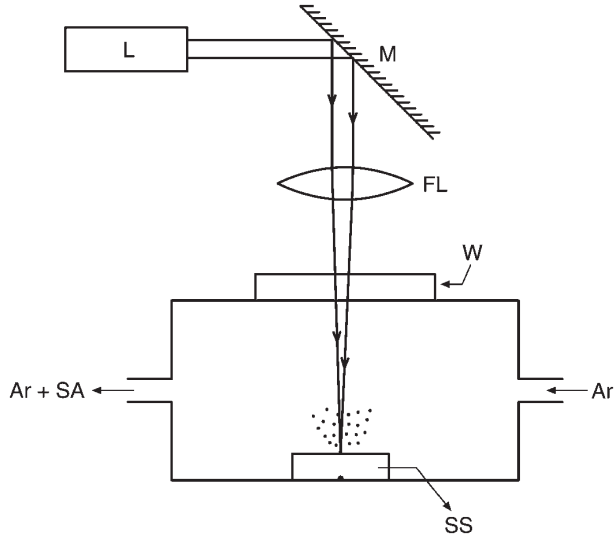


Figure 7.5 *Schematic representation of LA technique. Ar, Ar flow; Ar + SA, Ar flow containing sample solid aerosol directed to plasma; L, laser source; FL, focusing lenses; W, optical window; SS, solid sample; and M, mirror*

wavelength on this exit slit. While the sample is continuously introduced into plasma, measurement on each selected line is made for a time period that is sufficient to give a good *S/N* value; multi-element measurement is thus realized in a *sequential mode*. In some instruments, the grating is stationary and the detector moves to an assigned position on the focal plane. Some ICP-OES instruments were made so that they were equipped with a polychromator and several exit slits and detectors at selected wavelengths to perform multi-element detection in a *simultaneous mode*; in these systems none of the optical components has to be moved. For some time the advantages and disadvantages of these two systems were under discussion. Sequential ICP-OES instruments had lower cost, flexibility in choice of analyte but they did lack the availability of fast multi-element analysis. On the other hand, simultaneous ICP-OES instruments cost more, but these are capable of producing large number of data in unit time; the selections for wavelength and therefore the chemical matrix to work on are limited and should be determined before the instrument is designed. Thus, simultaneous instruments with fixed exit slits lacked flexibility in wavelength and thus analyte and matrix. Particularly with advances in array detector systems such as charge transfer devices, *charge coupled device* (CCD) and *charge injection device* (CID), most ICP-OES instruments now have an Echelle monochromator with a two-dimensional focal plane (see Chapter 5 for Echelle monochromators) on which array detectors are mounted. Therefore, fixed exit slits and limitations in wavelength is a part of history; fast and efficient simultaneous multi-element ICP-OES instruments are in the service of analytical chemist. A typical simultaneous multi-element ICP-OES system is shown in Figure 7.6.

Another classification regarding the measurement mode has been related to the manner by which the ICP source is viewed. In the early designs, the ICP torch was

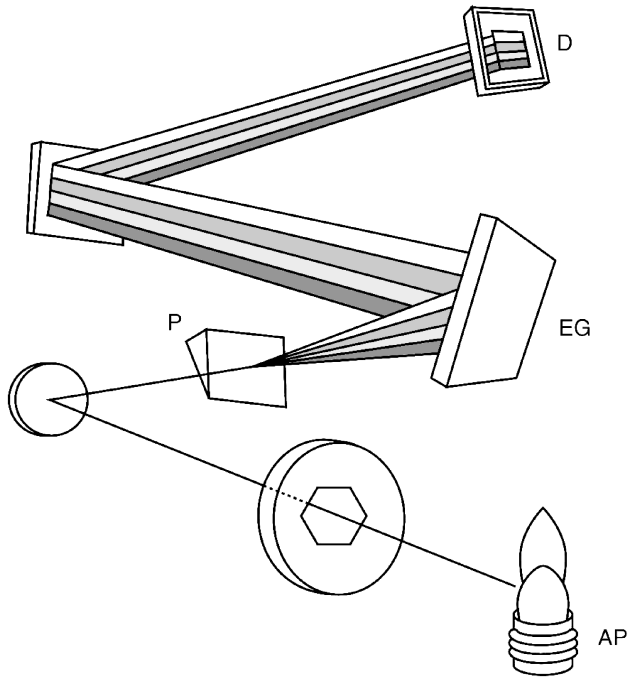


Figure 7.6 *A typical modern ICP-OES instrument with Echelle polychromator and charge injection device array detector, IRIS Intrepid II. AP, Argon plasma; P, 19° prism; EG, Echelle grating; and D, CID array detector (Adapted with permission from Thermo Electron Corporation, UK)*

mostly positioned in a vertical manner and a portion of the plasma was viewed radially by the entrance slit of spectrometer; this is presently called *side-on* or *radial viewing.* Later on, another mode of plasma viewing has become popular; in this sort of design, the plasma is positioned in a horizontal manner and is viewed along its long axis; the terms used for this sort of measurement are *end-on* or *axial viewing.* Using proper optical designs, an ICP source can be viewed radially or axially, irrespective of its position being vertical or horizontal. Some instruments provide both of these options; either can be chosen in a *dual view* design as shown in Figure 7.7. Axial viewing systems were used by researchers as early as 1970s; however, this technique became commercial in last 10 years. Axial viewing provides a sensitivity enhancement of typically 5–20 times since a longer source pathlength is viewed; but this system is more prone to non-spectral interferences. On the other hand, radial system is relatively more free of interferences although its sensitivity is lower.

7.2.2.4 Interferences

Interferences in ICP-OES can be classified as *spectral* and *non-spectral* in origin.

Spectral interferences on analyte emission signal are caused by emission generated by other atomic or ionic species or background emission by the source itself.

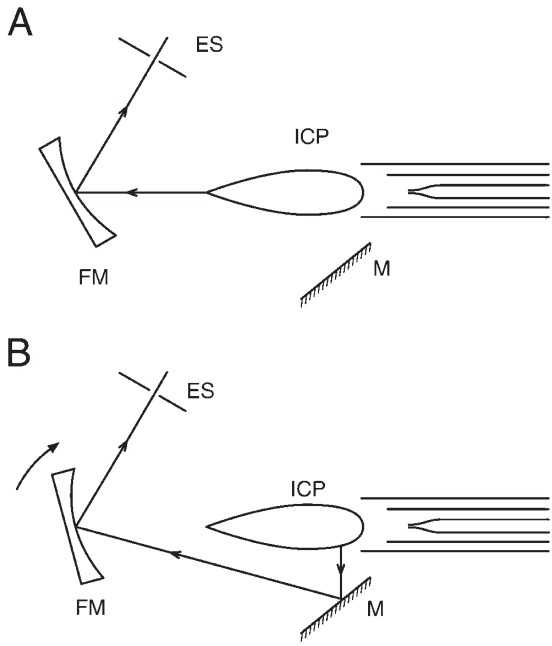


Figure 7.7 *Principle of dual view ICP-OES system, axial (A) and radial (B) modes. ICP, inductively coupled plasma; M, flat mirror; FM, focusing mirror; and ES, entrance slit of monochromator*

This sort of interference can be in the form of a sharp peak in case of an interfering atomic or ionic species or a straight, sloping or structured background. In case of line interference, if the difference in wavelength is less than 0.01 nm, separation may be difficult depending on the resolution of instrument. Choosing another analyte wavelength is the best solution. In any case a *spectral profile* in vicinity of analyte emission signal should be taken prior to analysis, using the matrix with analyte. After a careful investigation of the spectral profile, analyst should decide at which wavelength the background signal should be chosen for best spectral correction. As the spectral profiles are examined several possibilities with different analyte wavelengths should be considered; any signal profile with complex background should be avoided. In order to assure accuracy, another wavelength may be chosen where spectral interference is easier to deal with. In this process, a peak with lower sensitivity may have to be selected; sensitivity may be sacrificed for better accuracy. Some typical background signals and spectral profiles are shown in Figure 7.8.

Non-spectral interferences may be caused by sample transport and sample introduction problems. For aqueous solutions, peristaltic pumps are used to assure a constant sample flow to nebulizer; however, due to differences in solution viscosity nebulization efficiencies may not be the same for sample and standard. Standard addition or matrix matching techniques should be used to eliminate such errors.

Another source of non-spectral interference is the alterations in Ar plasma equilibria. Plasma conditions may be often affected by the presence of *easily ionizable*

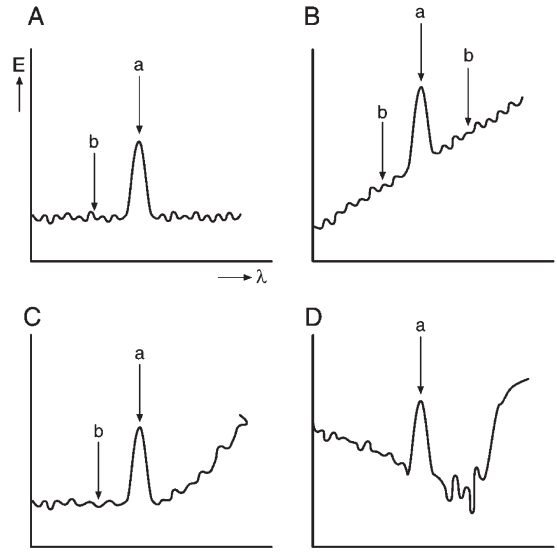


Figure 7.8 *Typical ICP-OES spectral profiles showing analyte and background signals. (A) Straight background, (B) sloping background (average value of two background values are used), (C) wing background, (D) structured background (to be avoided). a, Analyte wavelength; b, choice(s) of wavelength for background signal correction*

elements (EIEs) such as calcium and sodium. High amounts of these elements may have to be introduced into plasma as a result of the natural sample matrix or fusion methods used to dissolve solid samples. Presence of EIEs in relatively large quantities in plasma upsets equilibrium conditions and thus both suppressing and enhancing effects in complex ways may take place for analyte signals in ICP.

The presence of high amounts of solids in plasma may affect plasma conditions in many ways. The term *robustness* is used to express the capability of a plasma system to accept a change in concentration of major elements, acids and other species without any significant variations in line intensity of analyte. Robustness of plasma conditions can be verified by measuring the intensity ratio of an ionic to atomic spectral lines of the same element. In order to analyze the robustness conditions Mg II (280.270 nm)/Mg I (285.213 nm) intensity ratio has been used.[8,9] When the ratio is 10 or more, robustness is satisfactory. A ratio below 4 corresponds to high sensitivity to matrix effects. The robustness does depend on RF power on the system and thus can be optimized by altering this variable.

7.3 Inductively Coupled Plasma–Mass Spectrometry

Inductively Coupled Plasma–Mass Spectrometry is currently the most sensitive and powerful technique for trace element determinations. It is fast, multi-element and

very sensitive. Although its initial and running cost is relatively high, its high capacity for analysis and other positive properties has made this technique almost absolutely necessary in a trace analysis laboratory.

Before going into details of instrumentation and interferences, it would be useful here to briefly present the basic terms and principles of mass spectrometry (MS).

The number of *protons* is equal to number of electrons for a neutral element and mark the identity of an element; this is called *atomic number* (*Z*) or *proton number*. The total number of protons and neutrons for an element is called its *mass number* (*A*). Number of *electrons* determines the chemical behaviour of an element since chemical reactions are based on interaction of electrons. The common name for proton and *neutron* is *nucleon*; the former has a charge of +1 and the latter has no charge. The forms of an element with different number of neutrons in their nuclei are called *isotopes*. Most of the elements have more than one isotope. *Stable isotopes* occur naturally and the abundance of each particular stable isotope in earth crust is known. Abundances are shown as percentage values following the isotope, such as ^{74}Ge (35.94%). *Radioactive isotopes* do not have constant abundances; they undergo spontaneous disintegration that is called decay. The mass of an isotope is determined by its number of protons and neutrons, since electron has a negligible mass.

Atomic masses are represented in a relative scale where 1/12 of a single atom of ^{12}C is equal to one dalton (Da) or one atomic mass unit (amu). Since Avogadro number is 6.0221×10^{23} atoms mol^{-1} and 1 mol of ^{12}C is exactly 12.0000 g, it can be easily shown that 1 amu corresponds to 1.66054×10^{-24} g. Using the same scale, ^{80}Se and ^{208}Pb have atomic masses of 79.9165 and 207.9766 amu, respectively. *Nominal mass* values are often used in MS; ^{80}Se has a nominal mass of 80 amu.

As optical spectrometric techniques are based on handling and measuring light signals specific to analyte, the use of MS is based on generation of ionic species usually from a vapour of sample. The ions are accelerated and detected as based on their mass/charge ratio values in a high vacuum of 10^{-4}–10^{-8} torr. Several devices are used to separate species with different mass-to-charge ratios; these are called mass analyzers. The ions formed are mostly with a charge of +1, a smaller fraction has a charge of +2. In a rather less common form of MS, negative ions are formed; this is called *negative ion mass spectrometry*.

In MS measurements, mass number divided by the charge of the formed ion is called *mass to charge ratio* and denoted as *m*/*z*. A plot of signal intensity *vs*. *m*/*z* values is called a mass spectrum.

In a mass spectrum, although several criteria are used regarding the proper resolution of signals, the most used one is *abundance sensitivity* that is illustrated in Figure 7.9. The ratio of the maximum ion signal recorded at a mass *m* to the signal arising from the same species recorded at an adjacent mass of *m*+1 or *m*−1 is called *abundance sensitivity*. Using a quadrupole mass analyzer, resolution is sufficient for quantitative separation at a level of ±1 for *m*/*z*. As shown in Figure 7.9, abundance sensitivity at the lower and higher mass sides are not equal; the former has a lower abundance sensitivity. Actual typical abundance sensitivities for a quadrupole mass analyzer are in the order of 10^6–10^7; it should be noted that the associated values are much lower in Figure 7.9 for the sake of illustration.

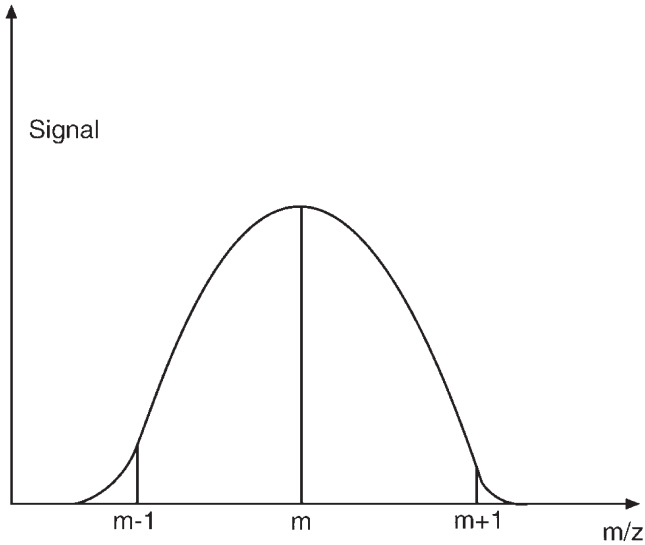


Figure 7.9 *Schematic representation of the concept of abundance sensitivity*

7.3.1 Instrumentation

A mass spectrometer consists of an *ion source*, a *mass analyzer* and a *mass detector*. In ICP-MS technique, the ion source is the argon plasma.

7.3.1.1 Mass Analyzers

ICP-MS is the combination of an argon plasma as the ion source and a mass spectrometer. Most mass spectrometers have been designed to determine molecules and thus are based on formation of molecular ions. There are many ways for formation and ionization of molecules in vapour form to be detected. The working principles of mass spectrometers require an operation in evacuated medium. In most of other mass spectrometers, ionization is carried out in vacuum. However, ambient conditions are required to form and sustain argon plasma. Therefore, the atomic ions mostly with a charge of $+1$ are formed in argon plasma at ambient pressure conditions and these should then be transported into a mass spectrometer operating in vacuum. Preservation of a high vacuum is difficult and any opening to accept ions from outside will cause a leak that will prevent proper evacuation. This technical problem has been a center of attention especially in the early years of the development of ICP-MS. The solution is uses an interface where the ions are first accepted to a section with a pressure that is between the ambient pressure and that of a high vacuum. A typical interface between argon plasma ion source and a quadrupole mass spectrometer is shown in Figure 7.10. The upper end of the plasma is in contact with the *sampling cone* in such a way that the flame-like tail is diverted out; this section is rather cooler as it contacts with ambient air and is not suitable analytically. A part of plasma products from a useful zone is allowed to enter the sampling cone into the

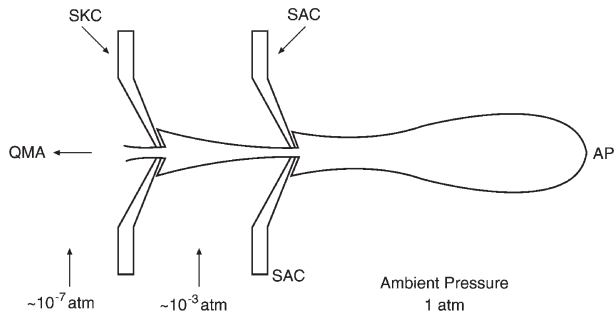


Figure 7.10 *Schematic representation of an interface between argon plasma and a quadrupole mass spectrometer. AP, Argon plasma; SAC, sampling cone; SKC, skimmer cone; QMA, quadrupole mass analyzer*

region of intermediate pressure. A second entrance is provided by *skimmer cone* into the high vacuum region where quadrupole mass analyzer (QMA) and detector are located. Both of these cones are made of nickel or platinum that are resistant to corrosion; they also have high heat conductance so that efficient cooling is feasible. Samples with high solid content may cause accumulation at orifices of both cones adversely affecting sensitivity; orifices have a diameter of about 1.0 mm.

The mostly used mass analyzers are *magnetic sector analyzer*, *quadrupole mass filters* and *time-of-flight* (TOF) systems. ICP-MS instruments with magnetic sector analyzer can reach very high resolving powers. Presently, the use of these instruments is not as common as ICP-MS instruments with quadrupole analyzers. Magnetic sector analyzer use a permanent electromagnet to cause the ion beam to travel a circular path, the species are spatially separated depending on their *m*/*z* values. Species with a selected *m*/*z* value are directed through an exit slit to detector. In this respect, this type of mass analyzer operates in a similar way to a monochromator that disperses the light into its wavelength components on a focal plane. By varying the current in the electromagnet, the magnetic field is also varied and species with different *m*/*z* values can be scanned on the exit slit.

QMA, on the contrary, behaves as a variable narrow band filter for species with different *m*/*z* values. The system consists of related electronics and four metal rods with a diameter of about 1 cm and a length of about 20 cm; these are made from stainless steel or molybdenum; most models are coated with a ceramic layer to prevent corrosion. Working principle is given in Figure 7.11. Four cylindrical metal rods are used as electrodes to allow the species with only a specified narrow range of *m*/*z* value. This is accomplished by applying DC voltage and RF voltage to rods in varying combinations. DC voltage is positive for two rods and negative in other two as shown in Figure 7.11. RF voltages applied to these pairs have same amplitude but different signs. The DC and RF voltage values are selected in such a way that in the corridor formed among these rods, the selected ions are directed to detector. While the selected ions with proper *m*/*z* value have a stable trajectory to pass the corridor, the others have unstable trajectories; these are either directed outside or collide to rods and are eliminated. Two of these rods behave as a low-pass mass filter, while

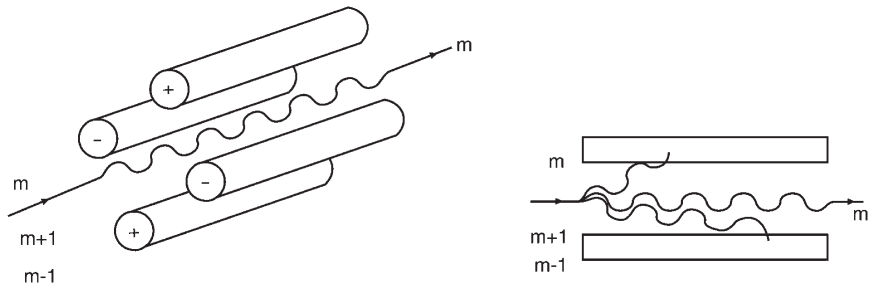


Figure 7.11 *Working principle of quadrupole mass analyzer. (m), (m+1) and (m–1) are m/z values that are separated*

the other two operate as the high-pass mass filter; a narrow band of *m*/*z* species are accelerated. As the DC and RF voltages are varied properly, scanning for different *m*/*z* values is accomplished. Using a quadrupole analyzer, scanning can be done in about 100 ms for a range of 1–240 for *m*/*z* values. In real operating conditions, however, several minutes are required to determine about 25 elements.

There are several measuring modes in ICP-MS instruments with QMA. One technique is *scanning* over *m*/*z* values. Single scan can be used for a fast pre-determination of matrix components so that strategies for interference minimization can be developed. Multiple scans at the cost of longer time can be used to obtain signals with better *S*/*N* values. Another mode of measurement is the *hopping mode*; this is to be used for samples of known matrix. In this mode, the time is allocated only for the measurements at the *m*/*z* value(s) of interest. Since the other (non-analyte) sections of mass spectrum is not measured, an evaluation of matrix components is not possible using this mode.

TOF-ICP-MS instruments are based on accelerating ions in a tube of about 1 m. The ions with lower *m*/*z* arrive at the detector at the end of the tube in a shorter time as compared to ions with higher *m*/*z* values. Therefore, the mass spectrum is obtained on the basis of signals from ionic species *vs*. time that corresponds to a certain *m*/*z* value. Since about 40 μs is required for scanning the whole *m*/*z* values, TOF is practically a simultaneous multi-element analyzer while QMA is a fast scanner. This is particularly useful when several elements are to be determined in a very small amount of sample causing just a transient signal. TOF-ICP-MS instruments are available for element determinations; however, their use is not yet as common as QMA.

7.3.1.2 Detectors

Electron multipliers are most commonly used in ICP-MS. The principle of ion detection is similar to that of PMT that was described in Chapter 5. The ions strike a sensitive metal plate that will then emit several electrons. The emitted electrons strike the next dynode that is held at a more positive voltage than the first one. Using several dynodes at successively more positive voltages, signal amplification is accomplished; these instruments have discrete dynodes. The detector may have a single continuous dynode, where the voltage gets progressively more positive along

the length of a curved surface inside a horn-shaped detector. Electron multipliers can provide a signal amplification of 10^5–10^8.

Among the other less commonly used detectors are *Faraday cup collector, photographic plates and scintillation-type transducers*.

7.3.2 Interferences

Interferences in ICP-MS can be classified as spectral and non-spectral in origin.

7.3.2.1 Spectral Interferences

Spectral interferences take place when the signal from an interferant overlaps with the analyte signal; the result is a positive error. QMAs have a satisfactory resolution of about 1 amu. Species with the same nominal mass will interfere with each other. Spectral interferences can be classified as follows:

- Isobaric interferences
- Doubly charged ion interference
- Polyatomic species interference
- Formation of oxides and hydroxides.

As an example for *isobaric interference* $^{96}Zr^+$, $^{96}Mo^+$ and $^{96}Ru^+$ will have overlapped signals in a mass spectrum as they will have the same *m*/*z* value 96. A list for selected isobaric interferences is shown in Table 7.1.

When the information given in Table 7.1 is examined, it can easily be observed that determination of nickel using ^{58}Ni (68.08) in steel is difficult because of interference from ^{58}Fe (0.28). Iron is an abundant element in nature; therefore, not only for steel but also in general the recommended isotope for nickel is ^{60}Ni (26.22) although its abundance and thus sensitivity is lower than that of ^{58}Ni (68.08). Similarly, determination of indium using ^{115}In (95.7) in a tin-rich matrix is not feasible; sensitivity is sacrificed for accuracy and ^{113}In (4.3) with significantly lower abundance than ^{115}In must be used. This is a good remedy, however, only if the sample does not contain significant cadmium at significant level. A full list of natural isotopes must be carefully examined and there should be sufficient information regarding the matrix composition of sample.

On the other hand, *doubly charged ions* are also formed in Ar plasma. Double charged $^{126}Te^{2+}$ ion with *m*/*z* value of 126/2 = 63 would spectrally interfere with mono charged ion of $^{63}Cu^+$ signal. Location in plasma is important for formation of M^{2+} species (Figure 7.12).

Most of the isobaric and doubly charged ion type interferences are caused by matrix elements in sample. One exception is the interference caused by $^{40}Ar^+$ (99.60%) during determination of $^{40}Ca^+$ (96.94%); this interference is caused by the main component of Ar plasma. In this case, as the most abundant isotope of calcium cannot be used, the ion formed by the second most abundant isotope, $^{44}Ca^+$ (2.09%), should be used at the cost of reduced sensitivity. It should be noted that if the matrix contains strontium, $^{88}Sr^{2+}$ (82.58) would interfere with $^{44}Ca^+$ signal.

Table 7.1 *Selected isobaric interferences in ICP-MS. Percent abundances shown in parenthesis have been taken from Ref. [10], rounded to two digits at most after the decimal point*

Analyte isotope	*Isobaric interferant(s)*
^{40}Ca (96.94)	^{40}Ar (99.60), ^{40}K (0.01)
^{48}Ti (73.8)	^{48}Ca (0.19)
^{50}Ti (5.4)	^{50}V (0.25), ^{50}Cr (4.35)
^{54}Fe (5.8)	^{54}Cr (2.37)
^{58}Ni (68.08)	^{58}Fe (0.28)
^{64}Zn (48.6)	^{64}Ni (0.93)
^{70}Ge (21.23)	^{70}Zn (0.6)
^{74}Ge (35.94)	^{74}Se (0.89)
^{76}Se (9.36)	^{76}Ge (7.44)
^{87}Rb (27.84)	^{87}Sr (7.00)
^{92}Mo (14.84)	^{92}Zr (17.15)
^{94}Zr (17.38)	^{94}Mo (9.25)
^{96}Mo (16.68)	^{96}Ru (5.52), ^{96}Zr (2.80)
^{113}Cd (12.22)	^{113}In (4.3)
^{115}In (95.7)	^{115}Sn (0.34)
^{122}Sn (4.63)	^{122}Te (2.60)
^{123}Sb (42.64)	^{123}Te (0.91)
^{138}Ba (71.70)	^{138}La (0.09), ^{138}Ce (0.25)
^{180}Hf (35.10)	^{180}Ta (0.012), ^{180}W (0.13)

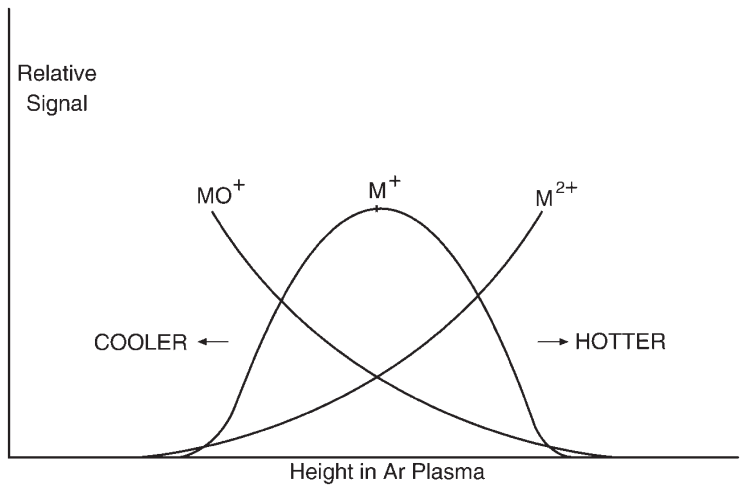


Figure 7.12 *Relative concentrations of MO^+, M^+ and M^{2+} along the height of Ar plasma (Adapted with permission from Thermo Electron Corporation, UK)*

A more serious type of interference is caused by the formation of *polyatomic and adduct species* formed in plasma, such as ^{40}Ar $^{16}O^+$ and ^{40}Ar $^{40}Ar^+$. This type of interference is caused by the species in plasma gas, solvent, matrix, atmosphere and reagents used in sample dissolution. In this respect, choice and concentration of

acid(s) used in sample dissolution becomes an important issue. Some possible sources for elements that can form polyatomic or adduct species are shown in Table 7.2. Selected interferences caused by polyatomic and adduct ion formation are given in Table 7.3.

Interferences caused by polyatomic, metal oxide and hydroxide ion formation has now been well documented in literature.[11] Some selected examples are given in Table 7.4.

7.3.2.2 Non-spectral Interferences

Non-spectral interferences take place when analyte concentration measured is altered for any reason; the result is positive or negative error. Most of these kinds of interferences take place during sample transport and introduction into plasma or by variations in plasma conditions. In order to minimize these type of interferences, it is necessary to take precautions to keep the plasma robustness and sample transport and introduction under control. It is desired in ICP-MS measurements that total dissolved

Table 7.2 *Some sources of elements to form polyatomic and adduct species causing interference in ICP-MS*

Element	*Possible source(s)*
Ar	Plasma gas
B	$LiBO_2$, $Li_2B_4O_7$ (fusion)
Cl	HCl, $HClO_4$
F	HF
H	Water, solvent, acids and organic sample matrix
N	Air, HNO_3, NH_3
O	Air, water, solvents, HNO_3, $HClO_4$, H_3PO_4 and H_2SO_4
P	H_3PO_4
S	H_2SO_4

Table 7.3 *Selected interferences caused by polyatomic and adduct ion formation in ICP-MS. Percent abundances shown in parenthesis have been taken from Ref. [10], rounded to two digits at most after the decimal point*

Analyte	*Interferant(s)*
^{42}Ca (0.65)	$^{42}Ar\ ^{2}H^{+}$
^{44}Ca (2.09)	$^{12}C\ ^{16}O^{16}\ O^{+}$
^{51}V (99.75)	$^{35}Cl\ ^{16}O^{+}$, $^{34}Cl\ ^{16}O^{1}\ H^{+}$, $^{14}N\ ^{37}Cl^{+}$
^{52}Cr (83.79)	$^{40}Ar\ ^{12}C^{+}$, $^{36}Ar\ ^{16}O^{+}$, $^{36}S\ ^{16}O^{+}$, $^{35}Cl\ ^{16}O^{1}\ H^{+}$
^{55}Mn (100)	$^{40}Ar\ ^{14}N^{1}\ H^{+}$
^{56}Fe (91.72)	$^{40}Ar\ ^{16}O^{+}$
^{63}Cu (69.17)	$^{31}P\ ^{16}O_2{}^{+}$
^{64}Zn (48.6)	$^{31}P\ ^{16}O^{16}\ O^{1}\ H^{+}$, $^{32}S\ ^{16}O^{16}\ O^{+}$, $^{32}S\ ^{32}S^{+}$
^{75}As (100)	$^{40}Ar\ ^{35}Cl^{+}$

Table 7.4 *Selected interferences caused by ions of metal oxides and hydroxides. Percent abundances shown in parenthesis have been taken from Ref.[10], rounded to two digits at most after the decimal point*

Analyte	*Interferant(s)*
^{43}Ca (0.14)	^{27}Al ^{16}O^{+}
^{56}Fe (91.72)	^{40}Ca ^{16}O^{+}
^{59}Co (100)	^{43}Ca ^{16}O^{+}, ^{42}Ca ^{16}O^{1} H^{+}, ^{40}Ar ^{18}O^{1} H^{+}
^{63}Cu (69.17)	^{47}Ti ^{16}O^{+}
^{66}Zn (27.9)	^{50}Ti ^{16}O^{+}
^{75}As (100)	^{59}Co ^{16}O^{+}, ^{43}Ca ^{16}O^{16} O^{+}
^{109}Ag (48.16)	^{92}Zr ^{16}O^{1} H^{+}
^{134}Ba (2.42)	^{102}Ru ^{16}O^{16} O^{+}
^{169}Tm (100)	^{153}Eu ^{16}O^{+}

solids in the sample solution be lower than 0.2%. Higher amounts will cause deposits in sampling and skimmer cones, lowering sensitivity. In addition, longer washout times are needed to prepare sample introduction system for next sampling. High amounts of solids also affect plasma conditions; this will also alter the analyte signal since plasma equilibria for ionic species will be affected. It is often required that the sample solution is diluted. For the reasons above, diluted samples are easier to handle. This approach will result in reduction of sensitivity; in addition, water blanks should be low and under control. However, since ICP-MS is a very sensitive technique, in most cases successful results can be obtained by this approach in despite of reduced concentrations due to dilution. On the other hand, sample introduction systems should also be under a good control. Lack of precision and accuracy in sample transportation is often a major source of error. Peristaltic pumps should be working well and other sample introduction systems should be in good condition.

7.3.2.3 Approaches for Elimination of Interferences

Several approaches are used to eliminate interferences in ICP-MS.

- Using another isotope of analyte
- Correction by computation
- Elimination of solvent
- Reaction/collision cells.

Naturally, one solution is to use another isotope of analyte that is not affected by the spectral interference; this is usually done at the cost of reduced sensitivity because of lower abundances.

When the spectral interference is caused only by the species that are not in sample matrix, blanks may be used for correction as long as their signals are not very high.

Problems caused by isobaric and doubly charged species can also be corrected by computation. If the only cause of interference is an isobaric isotope, intensity of

another isotope of interfering element is measured; since signals would be proportional to respective abundances of isotopes, the signal caused by interfering isotope is computed and subtracted from the total signal at analyte mass. Similar computations can also be carried out for interference caused by doubly charged ions.

Presence of solvent in aerosol introduced to plasma has several adverse effects. First of all, solvent is a potential source of elements that will form interfering species as shown in Table 7.2. Second, solvent cools the plasma, affecting equilibrium of atoms and ions. It is therefore desirable to remove solvent prior to transport of sample into plasma; in other words, it is nearly ideal to introduce sample in the form of a *dry aerosol* to plasma. Techniques such as LA produces dry aerosols and are therefore advantageous for solid samples.

In order to eliminate solvent prior to introduction to Ar plasma, several approaches may be used. Ultrasonic nebulizers usually have online solvent removal systems. After nebulization, the aerosol is passed through a heated zone; solvent evaporates here. In the next stage, the transport tube is cooled and solvent is separated by condensation; dry aerosol is thus formed to be introduced to plasma. In some systems, wet aerosol is passed through a tube surrounded by a non-polar membrane such as PTFE; organic solvents pass through this membrane and pumped out by a flushing argon stream. This technique is called *membrane desolvation*; it is schematically shown in Figure 7.13. Using membrane desolvation, losses may take place if analyte is in the form of organometallic compound with low polarity.

Use of a *reaction cell* or *collision cell* is another effective way to eliminate spectral interferences. The principle of a reaction/collision cell is to selectively affect and prevent the formation of interfering species. This is done in a section prior to mass analyzer. Gases such as NH_3, H_2 and He are used. As the interfering compounds are converted to new species that will not interfere, the formation of analyte ions are not affected. In case of NH_3, this molecule reacts with Ar^+ (products are NH_3^+ and Ar); formation of Ca^+ is not affected since the reaction involved is very slow. Therefore, interference of $^{40}Ar^+$ (99.60) on $^{40}Ca^+$ (96.94) is reduced or eliminated. Effect of collision cell on an aqueous solution containing a mixture of helium and hydrogen is illustrated in Figure 7.14. Interfering species such as $^{40}Ar\ ^{16}O^+$ for ^{56}Fe (91.72)

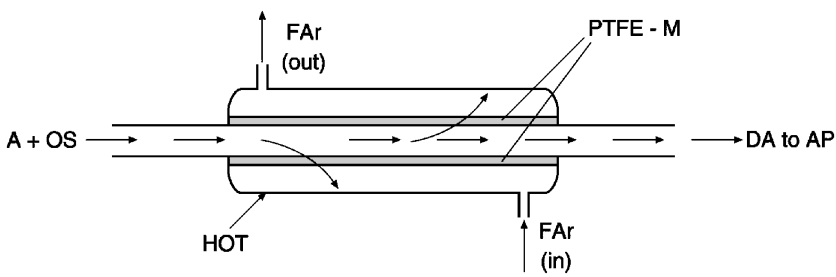


Figure 7.13 *Schematic representation of principle of membrane desolvation to produce dry aerosol. A+OS, aerosol + organic solvent; FAr, flushing argon; HOT, heated outer tube; PTFE-M, polytetrafluoroethylene membrane; DA, dry aerosol; and AP, argon plasma*

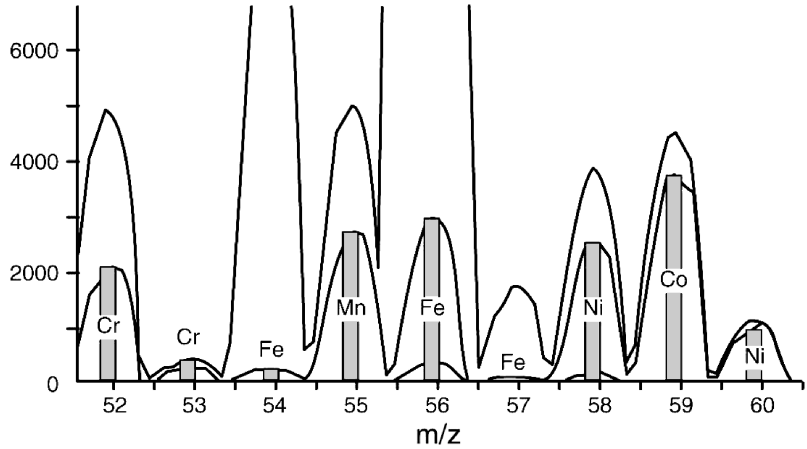


Figure 7.14 *Effect of collision cell to remove spectral interferences. Higher trace for signals were taken without using collision cell. Lower trace shows signals when collision cell was used with 93% helium and 7% hydrogen. (Adapted with permission from Thermo Electron Corporation, UK)*

and $^{40}Ar\ ^{12}C^{+}$ for ^{52}Cr (83.79) are converted to hydrogen or helium compounds of Ar; spectral interferences are eliminated. Reaction/collision cells are now an integral part of ICP-MS instruments.

Non-spectral interferences require precautions to keep the plasma robustness and sample transport under control. Regarding plasma conditions, diluted samples are easier to handle.

Handling interference problems in ICP-MS measurements requires that a thorough information regarding sample composition is available. Actually, this statement is meaningful for any kind of trace element analysis. However, in case of ICP-MS analysis, knowledge, intuition and proper remedies are very important, as this technique is presently the most sensitive one in its field. Dealing with low analyte levels also require cleaner laboratory environment, water and reagents of high purity.

7.3.3 Isotope Dilution ICP-MS

Isotope dilution (ID) technique is probably the most trusted analytical approach for its precision and accuracy. Its roots are in the early applications of radiochemistry. ID technique has become more applicable after invention of ICP-MS. During initial characterization of a certified reference material, it is desired that at least few laboratories present their results by using ID-ICP-MS.

The technique is based on the measurement of isotope ratios for analyte element in the original solution (sample, s), spike solution (tracer, t) and the spiked sample solution (mixture, m). The analyte element must have at least two stable isotopes (1) and (2). The samples will contain the isotopes (1) and (2) according to their natural abundances. Tracer solution is enriched in one of the isotopes; for instance (2). Sample

volume and/or weight is accurately measured in the beginning of analysis as usual. A known amount of the spike solution is added to the sample taken. The resulting spiked solution is mixed thoroughly to assure homogeneity. After this step, it may be necessary to carry out some chemical procedures such as precipitation, complexation, extraction, separation, *etc*. Since both the isotopes (1) and (2) have the same chemical behaviour, in case of any loss their ratio will not be altered. The calculations made are as follows.

The sample, tracer (spike) and mixture solutions will have the subscripts of s, t and m, respectively; the isotopes (1) and (2) will also be denoted by their respective numbers. The number of moles will be shown such as (^2n_t), meaning the number of moles of isotope (2) in the tracer (t) solution. R_s, R_t and R_m are the respective values for the measured isotopic ratios of isotope (1) to isotope (2). The molar sensitivities ^1S and ^2S are the number of detected ions per second per mole for each isotope. Ideally, ^1S and ^2S have the same values. Naturally, the isotopic ratio values will have the following relation when isotope (2) is the enriched species:

$$R_t < R_m < R_s \tag{7.1}$$

For the sample (s), tracer (t) and mixture (m) solutions the following requations can be written:

$$R_s = {}^1S\,({}^1n_s) \,/\, {}^2S\,({}^2n_s){}^1S\,({}^1n_s) = R_s\,{}^2S\,({}^2n_s) \tag{7.2}$$

$$R_t = {}^1S\,({}^1n_t) \,/\, {}^2S\,({}^2n_t){}^1S\,({}^1n_t) = R_t\,{}^2S\,({}^2n_t) \tag{7.3}$$

$$R_m = {}^1S\,({}^1n_s + {}^1n_t) \,/\, {}^2S\,({}^2n_s + {}^2n_t) \tag{7.4}$$

Equations (7.2) and (7.3) are substituted into Equation (7.4).

$$R_m = R_s\,({}^2n_s) + R_t\,({}^2n_t) \,/\, {}^2n_s + {}^2n_t \tag{7.5}$$

Number of moles of the enriched isotope (2) in the sample can thus be calculated as follows:

$$^2n_s = {}^2n_t\,(R_m - R_t) \,/\, (R_s - R_m) \tag{7.6}$$

In this relation, R_s, R_t and R_m are the measured count rate ratios from ICP-MS experiments; ^2n_t is the known added number of moles for isotope (2). Abundances of natural isotopes are usually known with high accuracy for natural samples, except for Li, B and Pb. The reason is that some isotopes of these elements, *e.g.* ^{206}Pb, are products and thus indicative of natural decay of U and Th radioactive series at the sample site. Using θ_2, abundance for isotope (2) and M, molar mass for the analyte element, the total mass of analyte in the sample, M_s can be calculated.

$$M_s = (M/\,\theta_2)\,{}^2n_t\,[(R_m - R_t) \,/\, (R_s - R_m)] \tag{7.7}$$

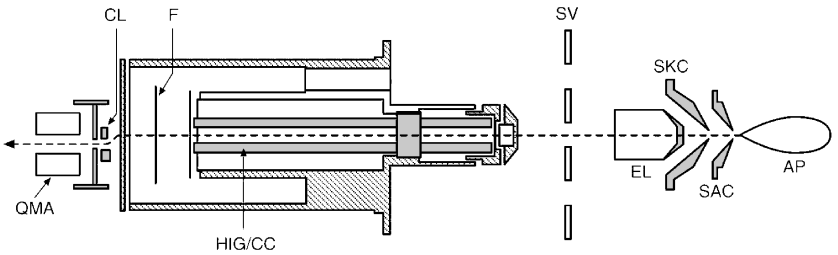


Figure 7.15 *Schematic representation of an ICP-MS instrument, Thermo Electron X Series. AP, Argon plasma; SAC, sampling cone; SKC, skimmer cone; SV, slide valve; EL, extraction lens; HIG/CC, hexapole ion guide/collision cell; F, focus; CL, chicane lens; and QMA, quadrupole mass analyser (Adapted with permission from Thermo Electron Corporation, UK)*

The values of R_s, R_t and R_m do not depend on the mass or volume of the measured samples; M and θ_2 are constants; $({}^2n_t)$ is accurately measured in beginning. In order to obtain a precise value for M_s, each R value must have sufficient precision, this can be reached by measuring counts as large as practically feasible. On the other hand, the differences between the counts $(R_m - R_t)$ and $(R_s - R_m)$ should be maximized so that M_s will have the smallest standard deviation according to equations of error propagation. RSD values between 0.1% and 0.5% are often obtained by using ID-ICP-MS. The results obtained are not affected by any losses during the chemical steps prior to ICP-MS measurements. In addition, any effects of signal enhancement or suppression are not important since the ratio of counts for isotopes (1) and (2) will remain constant. Therefore, the technique is immune to many kinds of error. In case of very low analyte concentrations, however, any analyte contamination prior to ICP-MS measurement will induce errors by altering the value of R_m. The cases in which R values are closer to 1.00 will be much less affected by any contamination.

7.3.4 Instruments and Applications

A typical ICP-MS instrument is schematically shown in Figure 7.15. Most of ICP-MS instruments are now equipped with efficient quadrupole mass analyzers and either collision or reaction cells are required to minimize spectral interferences. The basic consumables for an ICP-MS instrument are argon gas, sampling and skimmer cones and torches. These instruments are highly capable of element determinations rapidly, safely and with low-detection limits.

Regarding food and diet analysis, matrices are rather simple and mostly organic unlike the samples with very heavy metal or inorganic matrices as those in metal and mining industries. Most of the organic matrix in food is destroyed during dissolution and decomposition steps. Therefore, the basic concern regarding spectral interferences are related to the choice of acid and its remaining concentration in the final solution. Regarding interference problems, high sensitivity of the technique is another advantage because dilution is allowed to minimize the matrix effects.

References

1. S. Greenfield, C.T. Berry and I.L. Jones, *Analyst*, 1964, **89**(106), 713.
2. R.H. Wendt and V.A. Fassel, *Anal. Chem.*, 1965, **37**(7), 920.
3. L. Gray, *Analyst*, 1975, **100**(1190), 289.
4. J.M. Ottaway and F. Shaw, *Analyst*, 1975, **100**(1191), 438.
5. J.M. Ottaway, L. Bezur and J. Marshall, *Analyst*, 1980, **105**(1256), 1130.
6. R.F. Browner and A.W. Boom, *Anal. Chem*., 1984, **56**, 786A.
7. T.R. Smith and M. Bonner Denton, *Appl. Spectrosc.*, 1990, **44**(1), 21.
8. J.M. Mermet, *Anal. Chim. Acta*, 1991, **250**, 85.
9. J.M. Mermet and E. Poussel, *Appl. Spectrosc*., 1995, **49**(10), 12A.
10. D.R. Lide (ed), *CRC Handbook of Chemistry and Physics*, 76th edn, CRC Press, Boca Raton, 1995/1996, (1–10)–(1–12).
11. T.W. May and R.H. Wiedmayer, *Atom. Spectrosc*., 1998, **19**(5), 150.

Further Readings

1. A. Montaser and D.W. Golightly (eds), *Inductively Coupled Plasmas in Analytical Atomic Spectrometry*, 2nd edn, VCH Publishers Inc., New York, 1992.
2. P.W.J.M. Boumans (ed), *Inductively Coupled Plasma Emission Spectrometry*, *Part I*, *Methodology*, *Instrumentation and Performance*, *Chemical Analysis Series*, Vol. 90, Wiley, New York, 1987.
3. P.W.J.M. Boumans (ed), *Inductively Coupled Plasma Emission Spectrometry*, *Part II*, *Applications and Fundamentals*, *Chemical Analysis Series*, Vol. 90, Wiley, New York, 1987.
4. L.H.C. Lajunen, *Spectrochemical Analysis by Atomic Absorption and Emission*, The Royal Society of Chemistry, Cambridge, 1992.
5. J.R. Dean, *Atomic Absorption and Plasma Spectroscopy*, 2nd edn, *Analytical Chemistry by Open Learning*, Wiley, Chichester, 1997.

CHAPTER 8

Atomic Fluorescence Spectrometry

8.1 Introduction, History and Principles

Analyte atoms can be excited to upper electronic states by absorption of photons from a radiation source, relaxation to lower levels results in emission of some photons; this process is called *atomic fluorescence* (AF). The phenomenon of AF has been known since late 19th century; fluorescence signals from several atoms, such as Na, Hg and Cd, were observed in flames. In the early years of studies on AAS, a new interest was initiated on AF; Alkemade suggested its use for chemical analysis in 1956; Winefordner and Vickers realized true chemical measurements using *atomic fluorescence spectrometry* (AFS). Since then, and especially with the use of laser sources for excitation, AFS has been an important subject of study for the researchers in the field of atomic spectrometry. However, the acceptance of the technique in the form of commercialized instruments could not be realized to the same extent as research studies. Despite this very low level of acceptance by the users, active research continues on AFS because of its high sensitivity in elemental analysis.

There are several types of AF as shown in Figure 8.1; the resulted spectra involve lines only. When the wavelengths of excitation and emission are equal, *resonance fluorescence* is observed as the both types shown in Figure 8.1a. The wavelengths of excitation and emission are not the same in many occasions; for these cases of *non-resonance fluorescence*, the terms *stokes* or *anti-stokes* are used when the excitation wavelength is shorter or longer than the emission wavelength, respectively. *Direct-line fluorescence* results if the same upper level is involved in both the excitation and emission processes (Figure 8.1b). On the other hand, if different upper levels are involved in excitation and emission, the term *stepwise fluorescence* is used (Figure 8.1c). If the radiational excitation is followed by thermal excitation, it is called *thermally assisted fluorescence* (Figure 8.1d). When two or more photons are used in exciting the analyte atoms to an upper level, *multi-photon fluorescence* results (Figure 8.1e). For all the kinds of AF mentioned above, the analyte atoms to absorb radiation may also be in an excited state; in this case the term *excited state fluorescence* is used so that both the upper and lower levels are in excited states. In the process of *sensitized fluorescence*, the photons from the light source are first

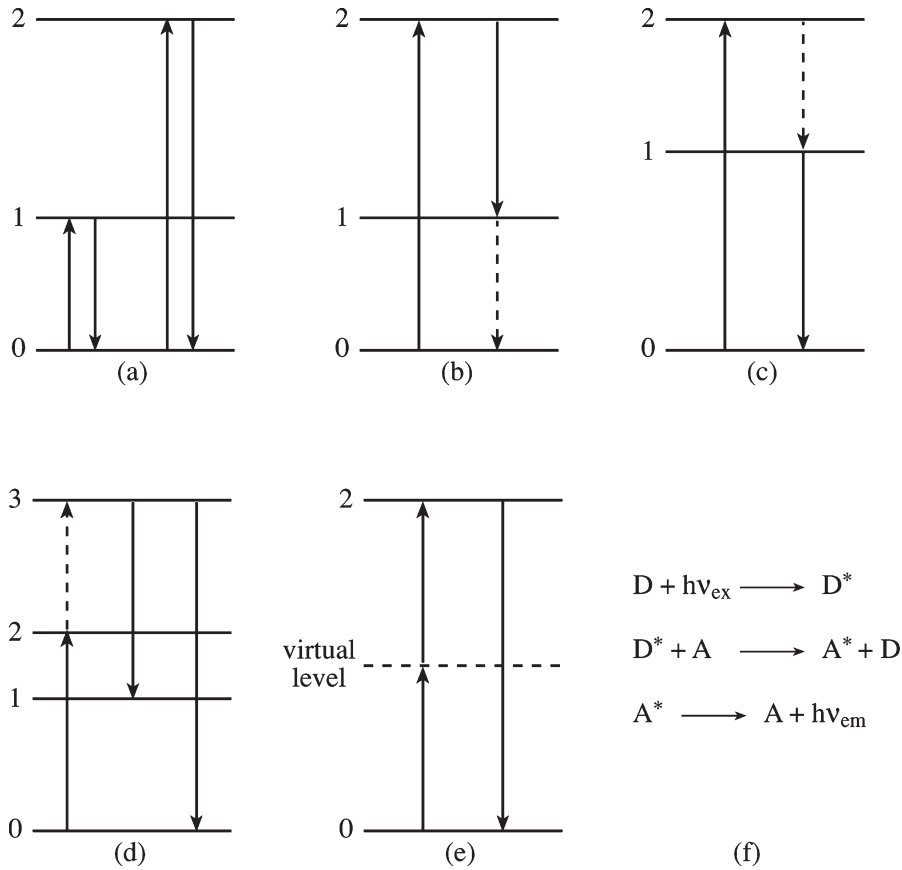


Figure 8.1 *Some common types of energy transitions in AFS. Full lines involve photons; dotted lines are for the radiationless transitions. (a) Resonance fluorescence; (b) direct-line fluorescence; (c) stepwise fluorescence; (d) thermally assisted fluorescence; (e) multi-photon fluorescence (for the case in the figure only two photons are involved and (f) sensitized fluorescence. D: donor; A: acceptor; *denotes the excited states; $h\nu_{ex}$ and $h\nu_{em}$ are photons for excitation and emission, respectively*

absorbed by a donor species D; the excited donor D* transfers its energy to an acceptor A; finally the excited state A* emits fluorescence as it relaxes (Figure 8.1f). Sensitized and multi-photon AF produce low radiational power; thus they are not analytically important. The resonance fluorescence is commonly used. The non-resonance fluorescence techniques are also used; since the wavelengths for excitation and emission are different, scattering problems are minimized.

The relation between the power of fluorescence emission and analyte concentration is given below in a simplified form. All the parameters are for a specific wavelength of measurement.

$$F = k\Phi P_0 2.3\varepsilon bC \tag{8.1}$$

F is the power of fluorescence emission; k the geometrical factor representing the efficiency of optical collection for both the exciting beam on atom cloud and the emission beam on the detector; Φ the *quantum yield*, the efficiency of the fluorescence process as compared to absorption; P_0 the radiational power of the excitation source; εbC the absorbance as given by Beer's law, the terms referring to absorptivity, pathlength and molar concentration, respectively; Φ, ε and c the intrinsic properties of analyte; and k, b and P_0 the instrumental parameters. For most of the experimental conditions, where P_0 is below the saturation level, the Equation (8.1) above describes a linear calibration plot when F is measured against concentration of analyte. If P_0 is excessively increased, the rates of absorption and deactivation becomes equal; this corresponds to a situation where the photons available for absorption are very high in number. In such a case, *saturation of fluorescence* is achieved, and the fluorescence signal does not increase with P_0 anymore; an example is shown in Figure 8.2.

The linearity of a calibration plot for a fluorescence measurement is affected by *self-absorption* where the emitted radiation is absorbed on its way to detector by the ground-state analyte atoms. For high analyte concentrations, this phenomenon causes a roll-over effect, so that a single F-value corresponds to two concentration values as shown in the Figure 8.3. If the atomic fluorescence signal was recorded by using aspiration to a flame atomizer in conventional manner, this situation is easily noticed as the steady-state signal will first have a peak higher than the rest of the signal. With a graphite furnace, or any sampling system producing a transient signal, it is difficult to realize whether the analyte has the lower or higher concentration value

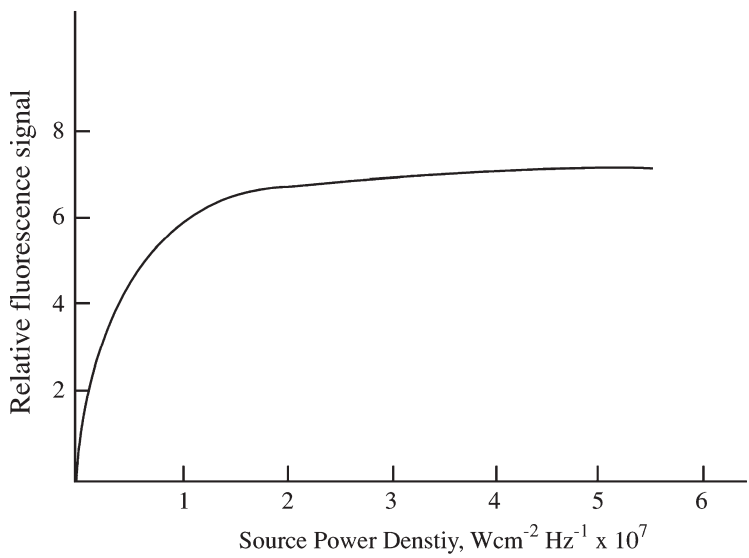


Figure 8.2 *Saturation of fluorescence for Tl in an air-H_2 flame. Laser excitation at 377.5 nm was used and the fluorescence emission was observed at 535.0 nm. The saturation takes place at 2.8 $\times$ 10^{-8} W cm^{-2} Hz^{-1}. (Adapted from Ref.1 with permission from Elsevier)*

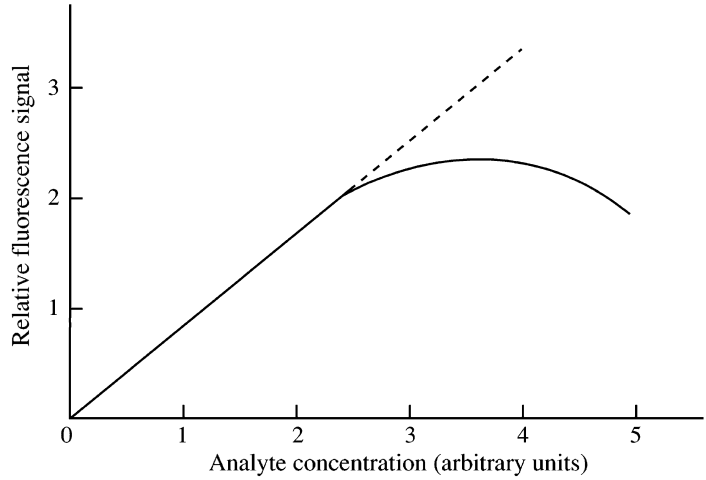


Figure 8.3 *Roll-over effect in AFS*

as shown on the calibration plot; measurement of another sample aliquot diluted by a different factor can solve the question.

8.2 Instrumentation

Several aspects are important in AFS instrumentation.

- Analyte species must be in the form of free atoms; a high, ideally complete degree of atomization is required. The atomizer must have a design and temperature to provide these conditions.
- The analyte atoms must be radiated by an exciting radiation source; its power P_o should be as high as possible up to saturation point (Figure 8.2), since the fluorescence emission power is directly proportional to P_0.
- A powerful excitation source causes a high degree of scattering by the constituents in the atomizer. In addition, many atomizers have their own background emissions. Therefore, the optical and electronic design must be able to handle high background signals. The choice of fluorescence emission wavelength is to be made in such a way that minimum background problems should be encountered.
- The excited atomic species may lose their energies through collisions with other constituents in the atomizer; this is called *quenching*. The environment of analyte atoms should have a composition that would cause minimum quenching.
- Unless special designs are used, the fraction of the fluorescence emission measured may be as small as 10^{-6} of the total radiation. The optical axes for excitation and emission are selected to have an angle of 90° between them; so that the exciting beam is not viewed; other angles except 180° can also be used. A maximum volume of the atom cloud must be subjected to the exciting beam and a maximum volume of the fluorescing part must be viewed by the detection system.

8.2.1 Excitation Sources

AF measurements have the high wavelength selectivity in a way similar to AAS when excitation is performed with a line source; the species may emit fluorescence within the spectral slit used will not be excited and thus will not interfere. When a continuum source is used for excitation, on the other hand, this advantage will be lost; however, multi-element detection may then be possible. Both the line and continuum sources have been used for AFS. Hollow cathode lamps (HCL) result in higher fluorescence intensity if they are pulsed. Special HCLs operated with higher than normal current pulses were used. As compared to HCL devices, electrodeless discharge lamps (EDL) have higher emission power and thus cause better AFS signals; however, their use is limited only to some elements and their radiational power drift with time. Regarding the continuum sources, high power EIMAC® Xe arc lamps were also used; multi-element detection is feasible with a high-resolution polychromator such as an Echelle system. Since the wavelength selectivity is a function of the spectral slit, high resolution is compulsory with a continuum source. A low-cost monochromator as used in AAS is sufficient when a line source is used where the resolution is limited by the source emission linewidth.

In last few years, lasers became increasingly popular in AFS studies. Commonly, a tunable laser pumped by a more powerful laser is used for excitation. Saturation of fluorescence can be achieved with such a system. Working with saturation by lasers has several advantages. In addition to high sensitivity, AF signal becomes independent of the source power P_0, and also any fluctuations in its value. Despite their excellent optical properties, lasers could not be used in absorption spectrometry, because well-stabilized, drift-free source emissions could not be obtained from these devices. In an AFS measurement with laser excitation, this disadvantage of lasers may be eliminated since the fluctuations cannot be sensed by the analyte atoms which are already at saturation point. Better linearity is obtained as the ground-state atoms are limited in number, self-absorption is minimal. Low-detection limits obtained by lasers result in a large dynamic range, up to 10^8. The thin optical profile of the laser beam should be matched by the atomizer design; graphite furnace has been fairly popular for this purpose. Using frequency doubling by special crystals, excitation in UV is possible.

Another excitation source used is the *inductively coupled plasma (ICP)* into which the analyte species are aspirated. High emission radiated at specific wavelengths from ICP functions as a single- or multiple-element line source system.

8.2.2 Atomizers

An ideal atom cell should be considered together with the properties of the exciting source and the detection system; it should have the following properties.

- It should provide a high degree of atomization, 100% as ideal.
- The analyte species must have a fairly long residence time, so that a good *S*/*N* value can be obtained by exciting the same atoms using a pulsed source.
- The atomic cloud, the exciting beam and the area viewed by the detection system must have a good overlap. The atomizer design must have the appropriate dimensions for this condition to give the best AFS signals.

- The environment of analyte species must have a composition that would cause the minimum quenching effect for analyte. The order of quenching for some common species are $Ar < H_2 < H_2O < N_2 < CO < O_2 < CO_2$.
- The atomizer should have a minimum background emission at analyte wavelength.

Flames were the most popular atomizers in the early stages of AFS research. When H_2 is employed as fuel, the flame has low quenching properties and a low background emission. Despite these useful properties, H_2 flames have relatively low temperatures and thus can atomize only certain elements. O_2 is better than air as an oxidant, because N_2 absorbs the heat and lowers the atomizer temperature. Some AFS flame heads were designed in both laminar and cylindrical shapes often with a protective steam of Ar resulting in a *separated flame*, so that the adverse effects of air were eliminated. Air-C_2H_2 and N_2O–C_2H_2 flames were also used.

ICP has been another atomizer for AFS. Since temperatures as high as 6000 K can easily be obtained in ICP, an effective atomization can be realized and chemical interferences are minimized. At such high-temperature values, ionization is appreciable and fluorescence from some ions can also be observed. Light scattering interferences are also significantly lower as compared to flames. In addition, the inert Ar atmosphere provides lower quenching as compared to flame environments. Background emission is generally higher than in flames. The AF signal is viewed above the coil, at a height of about 50 mm, where the background emission is minimal.

Some atomization techniques used in AAS, such as the hydride generation and cold vapour method for mercury can also be employed for AFS. As a matter of fact, a very sensitive commercial AFS system for Hg determination is based on cold vapour formation, as shown in Figure 8.4; this instrument can be used to determine Hg at sub ng mL^{-1} range.[1]

Electrothermal atomizers offer several advantages for AFS measurements. The inert Ar atmosphere used in a graphite furnace provides low quenching. Atoms are

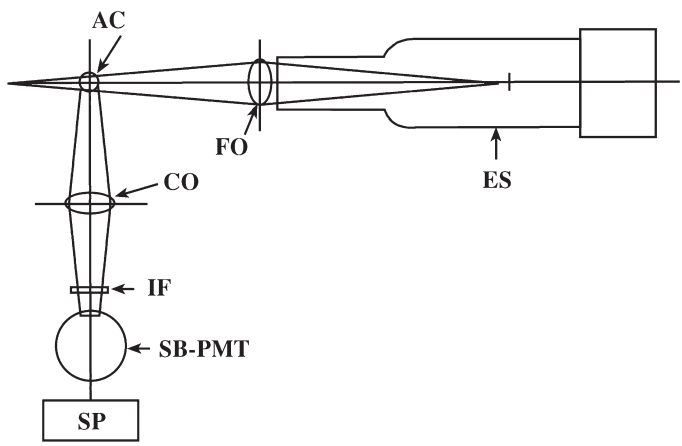


Figure 8.4 *Cold vapour Hg determination using AFS (Adapted with permission from PS Analytical Ltd, Orpington, Kent, UK); ES, excitation source; FO, focusing optics; AC, atom cell; CO, collimating optics; IF, interference filter; SB-PMT, solar-blind photomultiplier tube; SP, signal processor*

confined into a rather small volume for a fairly high-residence time; therefore a successful optical design for both the excitation and collection of emission can be conveniently realized when pulsed laser excitation is used. Although there is yet no commercial instruments, *laser-excited atomic fluorescence* (LEAF) has found a wide acceptance in research laboratories.

Most commonly, a hole is bored on the body of a graphite cuvette so that the axes for excitation and emission can be made in a 90° geometry. Due to the small-volume confinement and the presence of particles, light scattering in graphite furnaces is a serious problem. Usually non-resonance fluorescence modes are selected so that the scattering background near the excitation wavelength is avoided. The detection limits obtained with LEAFS is as low as ng L^{-1} or pg as absolute.

8.3 Interferences

Both spectral and non-spectral interferences are encountered in AFS measurements. Regarding non-spectral effects, all kinds of chemical interferences for AAS, affecting the free analyte atom concentration, are valid also for AFS. These will constitute multiplicative type of interferences. Chemical interferences minimized in an ICP atomizer, are fairly low in flames, but serious in graphite furnaces; standard addition technique should be applied whenever necessary. Spectral interferences are mainly due to background emission from atomizer, light scattering caused by particles and background fluorescence. The source modulation is used to eliminate the background thermal emission signal. In addition, emission from atomizers should be baffled so that a minimum portion of this signal should be received by detector. For the elimination of light scattering at the excitation wavelength, the most common approach is the use of non-resonance fluorescence. Wavelength selection for the fluorescence emission should be made such that minimum background emission and minimum scattering are encountered. The correction for background scattering can be made by using a line nearby the analytical wavelength; oscillating quartz plates and wavelength modulation have also been successfully used. Array detectors promise novel opportunities for background correction and multi-element detection, since simultaneous observation at all wavelengths in an interval is possible with these devices.

8.4 Instrumentation and Applications

Unfortunately, AFS did not become very common in trace analysis. Despite the general interest that is still alive in research laboratories, there are very few instruments for routine applications.

LEAF instrumentation is very popular in research laboratories studying on AFS. The instrumental setup from a recent work by Winefordner group[2] is shown on Figure 8.5. This system has been used for the determination of Pb in blood; with an absolute detection limit of 100 ag. A dye laser pumped by a copper vapour laser was used to excite Pb at 283.3 nm; the non-resonance fluorescence emission from the analyte in a graphite furnace was measured at 405.8 nm. Since the system has very

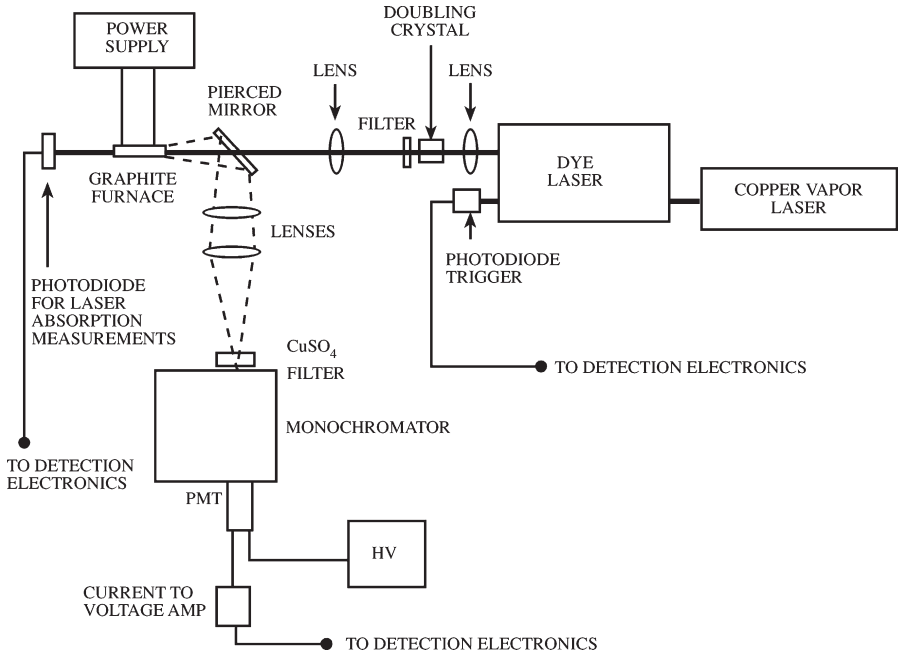


Figure 8.5 *Laser-excited atomic fluorescence spectrometry (LEAFS) instrument (Adapted from Ref. 2 with permission of American Chemical Society, Washington DC, USA)*

high sensitivity, a large dilution as 1:21 allowed determination without using standard addition or matrix modifiers.

In future, laser-excited AFS methods having high sensitivity may become more common if the cost of lasers that are capable of exciting atomic clouds could be available at significantly lower costs.

References

1. D.R. De Olivares and G.M. Hieftje, *Spectrochim. Acta*. 1981, **36B**, 1059.
2. E.P. Wagner, II, B.V. Smith and J.D. Winefordner, *Anal. Chem.*, 1996, **68**, 3199.

Further Reading

1. G.F. Kirkbright and M. Sargent, *Atomic Absorption and Fluorescence Spectrometry*, Academic Press, London, 1974.
2. J.D. Ingle Jr., S.R. Crouch, *Spectrochemical Analysis*, Prentice-Hall, New Jersey, 1988.
3. C. Vandecasteele and C.B. Block, *Modern Methods for Trace Element Determination*, Wiley, Chicester, 1993.

CHAPTER 9

Nuclear Activation Analysis

9.1 Introduction

Since 1936, when the neutron activation analysis (NAA) was originated by George de Hevesy and Hilde Levi in Denmark,[1] the technique has had a fascinating history of growth and application.

Initially, the technique was considered by analytical chemists to be only an interesting curiosity, rather than an approach that might have any real usefulness for analysis in the real world. In order to develop into a really useful analytical technique, NAA had to wait a while for the invention of high-flux source of neutrons, the nuclear reactor, Na(Tl) scintillation, and later germanium semiconductor detector for γ-ray detection.

In addition, impressive developments in sources of activation, in detectors, in electronics and in data handling have paved the way for this technique for application in nearly all the branches of science. The major reasons for these achievements were

(a) the inherent sensitivity for a large number of elements,
(b) its non-destructive character,
(c) its activation and counting efficiency being nearly independent of matrix composition and chemical form,
(d) well-defined theoretical foundation where all sources of systematic or random variation are identifiable, and
(e) its freedom of contamination after irradiation in trace analysis.

9.2 Basic Principles

Activation analysis is based on the principle that when a material is irradiated in a nuclear reactor with neutrons, particle accelerator or other suitable source, some of the atoms present in the material will interact with the bombarding particles and be converted into different isotopes of the same element or isotopes of different elements depending on the nature of the bombarding particles. In many cases, the isotopes produced are radioactive. If each different induced radioactivity can be distinguished or separated from all other radioactivity produced, the amount of each radioactivity is a measure of the quantity of the parent isotope present in the material.

9.2.1 Radioactive Decay

An element is said to be radioactive if the nuclei of its atoms keep disintegrating spontaneously, transforming thereby into a different nuclear species, emitting nuclear particles and/or radiation in the process. There seem to be about 2000 radionuclides known to date, but only 274 are stable. The rest are unstable, *i.e.* radioactive: they each undergo one or more successive decay steps with emission of particles and/or quantum ray until a stable nuclide is reached.

Radioactive decay is a statistical process: the nuclei change without affecting one another, each radionuclide having a characteristic decay probability. The moment of disintegration for a single atom of an unstable nuclide cannot be predicted. The probability of decay is a property of the nuclide and is therefore independent of the chemical and physical conditions.

9.2.2 Half-Life

Consider the simplest case where a radionuclide A is converted into a stable nuclide B by emission of particle x.

$$\mathrm{A} \rightarrow \mathrm{B} + \mathrm{x} \tag{9.1}$$

The number of atoms N decaying in unit time is

$$\frac{\mathrm{d}N}{\mathrm{d}t} = -N\lambda \tag{9.2}$$

where λ is the decay constant. Integrating within the limits 0 to t gives

$$N = N_0 \mathrm{e}^{-\lambda t} \tag{9.3}$$

where N_0 is the number of radioactive nuclei at time zero.

The exponential law states that in a given time interval, the same fraction of radioactive nuclei will always decay. The time during which a given amount of radionuclide will disintegrate to half of its original amount is defined as "half-life", $\mathrm{t}_{1/2}$.

$$N = N_0/2 = N_0 \mathrm{e}^{-\lambda \mathrm{t}_{1/2}} \tag{9.4}$$

which results

$$t_{1/2} = \ln_{2/\lambda} = 0.693/\lambda \tag{9.5}$$

The half-lives of the radioactive isotopes can vary from fraction of seconds to millions of years. If at a certain time the mass of the observed radionuclide contains N atoms, then its activity A can be written as

$$A = A_0 \mathrm{e}^{-\lambda t} \tag{9.6}$$

The logarithm of both sides of Equation (9.6) yields

$$\log A = \log A_0 - 0.434\lambda t \tag{9.7}$$

where A is the activity at time t and A_0 is the activity at zero time.

The units of radioactivity are the Curie (Ci) and the Bequerel (Bq). One Ci is defined as 3.700×10^{10} disintegrations per second (dps) and 1 Bq as 1 dps. If radioactivity or count rate is plotted as a function of time on log-linear graph paper, a straight line is obtained. From the slope of this line, the half-life can be determined directly (Figure 9.1).

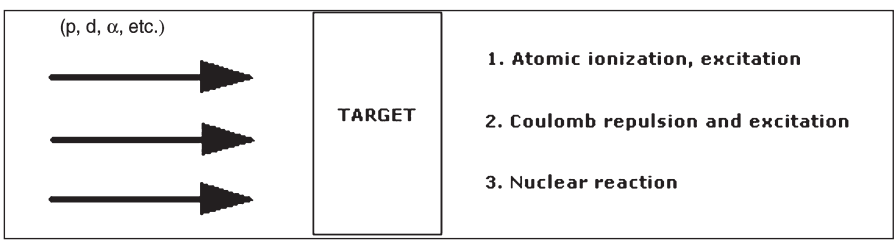
If two or more independent radionuclides are present in the same sample, the decay curve is a complex one. By analysis of this curve, the radioactivity or count rates of the individual radionuclides can be obtained. Nowadays, sophisticated computer programs can resolve many components with high precision.

9.2.3 Irradiation with Neutrons and Charged Particles

A reaction between a particle and an atomic nucleus is called an *induced nuclear reaction*. As a result of a nuclear reaction, one can form a new nucleus, or excite the original nucleus. These possibilities are dependent on the type of interacting particle, its energy and the type of target nucleus. As a result, a very large number of nuclear reactions with different properties may occur.

Charged particles such as proton (p), deuteron (d) and alpha particle (α) give rise to the following principal reactions with target nuclei:

Charged particles **Interactions with atomic electron**



In terms of nuclear activation analysis, nuclear transmutation reactions are the most important. As an example, let us consider the following reaction:

$$^{27}\mathrm{Al} + {}^{1}\mathrm{H} \rightarrow [{}^{28}\mathrm{Si}]^{*} \rightarrow {}^{27}\mathrm{Si} + {}^{1}\mathrm{n}$$

The intermediate compound nucleus ^{28}Si is bracketed to indicate its transitory nature and marked with* to indicate that it is in excited state. Induced reactions are, in general, written in a short form:

$$^{27}\mathrm{Al(p, n)}^{27}\mathrm{Si}$$

where p and n indicate incoming particle proton, and outgoing particle neutron, respectively.

As there is a Coulomb repulsion between the charged particle and target nucleus, we expect a so-called "Coulomb barrier" as indicated in Figure 9.2.

When the incoming charged particle is far away from the target nucleus, the kinetic energy of the projectile is not measurably affected by the Coulomb repulsion. As the projectile approaches the target nucleus, the Coulomb repulsion causes the potential energy to increase, as the kinetic energy of the particle decreases. A necessary condition is that charged particles must pass over the Coulomb barrier to cause nuclear reactions. If the projectile is a neutral particle, like a neutron, then, as seen in Figure 9.2(b), there will be no Coulomb barrier.

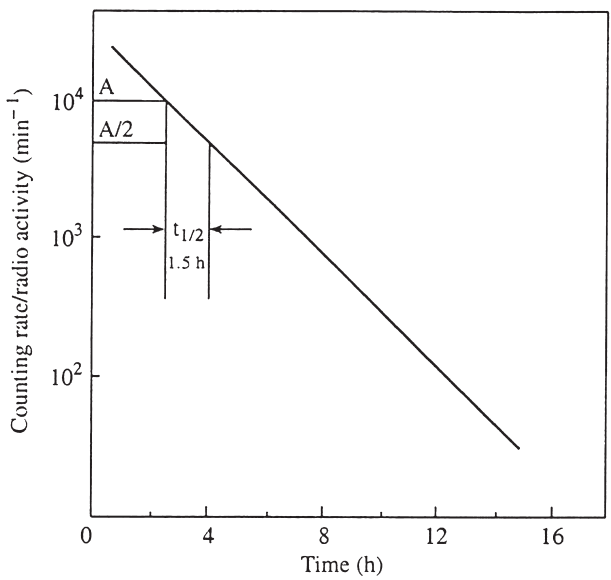


Figure 9.1 *Decrease of count rate or radioactivity with time. Half-life of the nuclide is determined as 1.5 h; A is activity, $t_{1/2}$ is half-life*

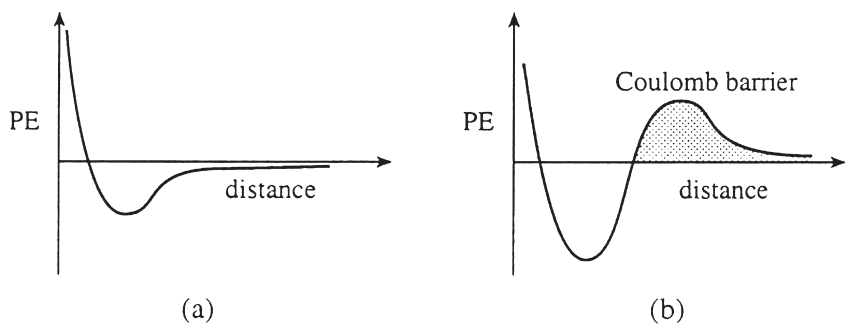


Figure 9.2 *(a) Potential-energy diagram for a neutron near a target nucleus. (b) Coulomb barrier for a charged particle near a target nucleus*

9.3 Neutron Activation Analysis

As there is no Coulomb barrier between neutrons and the target nucleus, the probability of having a nuclear reaction with neutrons is rather large as compared to charged particle reactions.

$$X + n \rightarrow [X+n]^{*} \rightarrow \text{Decomposition products}$$

target nucleus compound nucleus in excited state

The probability of the formation of a compound nucleus and subsequent mode of decay of the compound nucleus are dependent on the properties of target nucleus and energy of the neutron. For example, the irradiation of ^{35}Cl with neutron may result in a number of products:

$$^{36}Cl + \gamma$$

$$^{35}Cl + n$$

$$^{35}Cl + n \rightarrow ^{36}Cl^{*} \rightarrow ^{34}Cl + 2n + Q$$

$$^{35}S + p$$

etc.

where $^{36}Cl^{*}$ is the excited nucleus and Q is the reaction energy.

At very low neutron energies, thermal neutrons, $^{35}Cl(n, \gamma)^{36}Cl$ is the most probable reaction. With increasing neutron energies, one can have other reactions with different probabilities.

The most important nuclear reaction for NAA is the (n, γ) type, in which the excited nucleus decays to a lower energy state by the emission of "prompt" γ-rays. These prompt γ-rays with half-lives of 10^{-12}–10^{-16} s are also an important tool for identifying isotopes. This technique, prompt gamma activation analysis (PGAA) will be discussed later. The ground state of the excited nucleus will often be β^{-}, β^{+} or electron capture (EC) decay. The daughter nuclides will, in turn, emit, γ-rays. Half-lives associated with these γ-rays are dependent on the (n, γ) product half-life, which could be very short, in fraction of seconds, or very long, in years. Activation analysis using these delayed, γ-rays are commonly called "neutron activation analysis". The case of an (n, γ) reaction followed by β-decay is illustrated in Figure 9.3.

The velocities of neutrons and thus, their kinetic energies can differ widely. Being uncharged particles, neutrons cannot be accelerated; however, they can be moderated. Neutrons are generally classified according to their energies, En, in the following way:

$$\text{Fast neutrons: En} > 0.5 \text{ MeV}$$

$$\text{Slow neutrons: En} < 0.5 \text{ MeV}$$

Thermalized neutrons, corresponding to room temperature, 20 °C, have the velocity of 2200 m/s^{-1} which corresponds to 0.025 eV. The 0.2 eV neutrons are called *epithermal* and 1–300 eV neutrons are *resonance* neutrons.

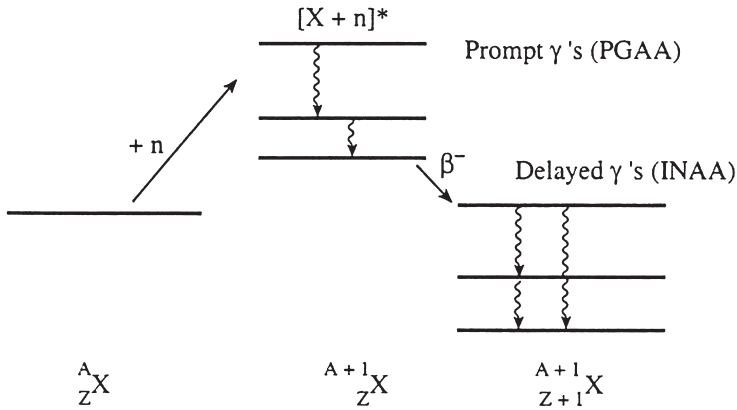


Figure 9.3 *(n, γ) reaction followed by β^- decay*

9.3.1 Cross Section

Considering size alone, the probability that the incident particles will strike the nucleus should be proportional to its cross-sectional area, πR^2.

Since $R = R_0 A^{1/3}$, then the area $= \pi R^2 = \pi R_0^2 A^{2/3}$. The R_0 is a constant, 1.2×10^{-13} cm. For mass number $A = 100$, the cross-sectional area will then be about 10^{-24} cm^2. The total cross section for collision with a fast particle is never greater than the geometric cross-sectional area of the nucleus. Therefore, fast particle cross sections are rarely much larger than 10^{-24} cm^2. Therefore, a cross section of 10^{-24} cm^2 is considered *as big as a barn*, and 10^{-24} cm^2 has been named the *barn*, which is the unit of cross sections.

$$1 \text{ barn} = 1 \text{ b} = 10^{-24} \text{ cm}^2 \text{ (definition)}$$

$$1 \text{ mb} = 10^{-3} \text{ b}$$

If we measure the cross section for neutron energies between 0.4 and 1 MeV, we see so-called *resonance peaks* at discrete energies for a given isotope. Except for very few isotopes, *e.g.*, Cd, Sm, Eu, Gd and Hg, the cross section changes as a function of $1/v$ up to 0.4 eV. This is called $1/v$ law where v is the velocity of neutrons. Above 1 eV, the cross sections show enormous fluctuations over a very small energy range due to resonance.

9.3.2 Neutron Sources

9.3.2.1 Laboratory Neutron Sources

These could be radioactive sources or neutron generators. In general, the main nuclear reaction for most laboratory sources is:

$$^{9}\text{Be}(\alpha, \text{n})^{12}\text{C}$$

Table 9.1 *Production of fast neutrons by various reactions*

Reaction	*Neutron Energy*
$^{9}Be(\gamma, n)^{8}Be$	Thermal, 0.025 eV
$^{9}Be(d, n)^{10}B$	4.4 MeV
$^{2}H(d, n)^{3}He$ ("DD" reaction)	0.3 MeV
$^{3}H(d, n)^{4}He$ ("DT" reaction)	14 MeV

DD: deuteron–deuteron reaction; DT: deuteron–tritium reaction

The αs can be obtained from the radioactive α emitters, such as Ra and Po. Spontaneously fissioning ^{252}Cf sources, which are now available in mg quantities, are becoming increasingly more important.

As seen in Table 9.1, fast neutron can be produced by bombarding certain nuclides with photons or charged particles.

Of these, the last one (DT) is most often used for the fast NAA.

9.3.2.2 Research Reactors

Research reactors are the main sources of thermal neutrons.

$$^{235}U + n \rightarrow {}^{236}U^{*} \rightarrow X + Y + 2.5n + Q$$

where X and Y are the fission fragments. An average of 2.5 neutrons is emitted during the fission of a ^{235}U atom.

The fission neutron spectrum shows a Maxwellian distribution ranging in 0–15 MeV, peaking at 1 MeV. Reactor neutrons are classified according to their energies into three groups: fast neutrons (fission spectrum), resonance neutrons and thermal neutrons. Fission neutrons are *moderated* for the propagation of the chain reaction to thermal energies.

9.3.3 Preparation of Samples for Irradiation

The experimental procedure for instrumental neutron activation analysis (INAA) vary greatly from laboratory to laboratory, depending on the type of irradiation facilities available, the counting equipment used, the elements to be determined, the type of sample and its matrix and the individual preferences of the experimenter. The flow diagram (Figure 9.4) shows an optimal sample preparation, irradiation and counting procedures to obtain as much information as possible.

Samples collected as explained in Chapter 4 should be unpacked and prepared in a particulate-free environment such as a "Class 100" clean room, or clean bench. A "Class 100" work area means an area where the air contains less than 100 particles per cubic foot. All the metal surfaces are painted with epoxy paint, and a system containing filters circulates the air rapidly in the room. Elemental standards, flux monitors or both must be positioned at known locations in the irradiation container.

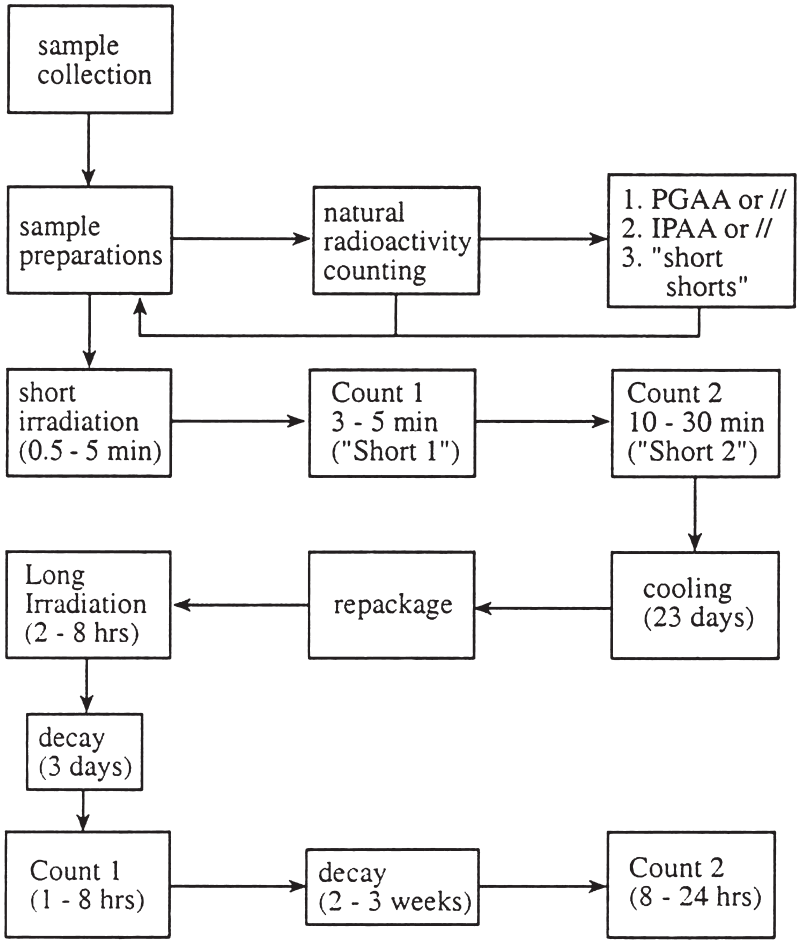


Figure 9.4 *Flow diagram for sample preparation, irradiation and counting procedure in activation analysis*

Prior to any irradiation, *samples* can be counted for natural activity as well as activities released to the environment by man-made sources. This aspect has become very important after the Chernobyl nuclear accident especially for food samples. Often, fission products can be measured, usually indicative of nuclear-weapon testing or reactor accidents. Debris from atmospheric tests may reach the stratosphere and return to the troposphere by storm action. Some isotopes of interest are listed in Table 9.2.

9.3.4 Short Irradiation

Extremely short irradiation (1–5 s) may sometimes be used to measure a number of isotopes, which have very short half-lives, such as ^{19}O, ^{20}F and ^{77m}Se (Table 9.3).

Table 9.2 *Isotopes for natural or man-made radioactivity counting*

Isotope	*Half-Life*	E_γ *(keV)*
^{7}Be	53.3 day	477.6
^{141}Ce	32.5 day	145.4
^{103}Ru	39.4 day	497.1
^{137}Cs	30.17 year	661.7
^{95}Zr	64.0 day	756.7
^{131}I	8.041 day	364.5
^{144}Ce	284.4 day	133.5
^{90}Sr	28.0 day	β^-

Table 9.3 *Isotopes and their properties measured in "short-shorts"*

Isotope	*Half-Life (s)*	E_γ *(keV)*
^{19}O	26.8	198.0
^{20}F	11.0	1634.0
^{28}Al	134.4	1779.0
^{46m}Sc	17.4	162.0
^{77m}Se	18.7	142.5

Counting times for γ-rays emitted from these isotopes usually are 10–30 s. If there are large amounts of sodium and/or aluminium in the sample, it may severely limit the use of this type of experiment because of high dead times and large Compton continua. The method developed by Chatt using Compton suppresser was very successful in measuring the selenium content of many Canadian vegetables and biological samples.[2]

For short irradiation, depending on the number of detectors available, 1–4 samples could be packed, separated and held in place by a strip of polyethylene foam. A nickel flux monitor (5–50 mg, depending on irradiation time) is placed in close proximity to each sample. Short-lived isotopes, used in first and second counts, their half-lives and γ-ray energies are given in Tables 9.4 and 9.5, respectively. Depending on the fast neutron flux and the amount of the Al and Si in the sample, ^{28}Al(n, p)^{27}Mg and ^{28}Si(n, p)^{28}Al reactions might interfere on Mg and Al, respectively. Also there might be an interference from 843.8 keV γ-ray of ^{27}Mg to 846.6 keV γ-ray of ^{56}Mn. Therefore one has to check all these and other possible interferences during the analysis of the γ-ray spectra. Fortunately available softwares make these corrections most of the time.

9.3.5 Intermediate and Long-Lived Isotopes, Long Irradiation

Samples that have been run for "shorts" are usually allowed to cool for several days prior to the re-irradiation. In many cases, it is preferable to completely re-bag the sample, if the contamination risk is small. In any case, suitable blanks must also be

Table 9.4 *Isotopes and their properties measured in short1*

Isotope	*Half-Life (min)*	E_γ *(keV)*
^{27}Mg	9.45	843.8
		1014.4
^{28}Al	2.24	1778.9
^{37}S	5.06	3103.3
^{49}Ca	8.72	3084.4
^{51}Ti	5.76	320.1
^{52}V	3.76	1434.2
^{66}Cu	5.10	1039.0

Table 9.5 *Isotopes and their properties measured in short2*

Isotope	*Half-Life (h)*	E_γ *(keV)*
^{24}Na	15.02	1368.5
		2753.9
^{38}Cl	0.620	1642.4
		2167.5
^{42}K	12.36	1524.7
^{56}Mn	2.576	846.6
		1811.2
^{69m}Zn	13.90	438.7
^{87m}Sr	0.291	388.4
^{128}I	0.417	442.9
^{139}Ba	1.388	165.8

prepared. For long irradiations, about 0.5% cobalt in aluminium wires is used as a flux monitor. Isotopes that are usually observed as a result of long irradiation in NAA of food and diet after 15–20 days cooling and after 1–2 months cooling are given in Tables 9.6 and 9.7, respectively.

9.3.6 Calculation of Activity Produced after Neutron Irradiation

Assume the simplest case: a thin foil or wire, containing n atoms, is irradiated for a period of time t, with neutron flux, Φ. If the production rate $R = \Phi\sigma n$, the rate of decay during the irradiation is

$$\frac{\mathrm{d}N}{\mathrm{d}t} = -\lambda N \tag{9.8}$$

then the rate of change of the number of nuclides is

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \Phi\sigma n - N\,\frac{\mathrm{d}N}{\mathrm{d}t} = R - \lambda N \tag{9.9}$$

Table 9.6 *Isotopes observable after 20 days of cooling in long irradiation (long1)*

Isotope	*Half-Life (days)*	E_γ *(keV)*
^{197}Hg	2.67	77.4
^{153}Sm	1.948	103.2
^{249}Np	2.355	106.4
^{99}Mo	2.758	140.5
		739.4
^{131m}Te	1.25	149.8
^{115}Cd	2.224	336.3
		527.9
^{198}Au	2.697	411.8
^{69m}Zn	0.575	438.7
^{140}La	1.68	487.0
		815.8
		1596.4
^{82}Br	1.47	554.3
		619.1
		776.5
^{76}As	1.096	559.5
		657.2
^{122}Sb	2.70	564.1
^{24}Na	0.6375	1368.5
^{42}K	0.515	1524.7

Table 9.7 *Isotopes observable after 1–2 months of cooling in long irradiation (long2)*

Isotope	*Half-Life (days)*	E_γ *(keV)*
^{75}Se	118.45	135.9
		264.5
^{141}Ce	32.55	145.4
^{131}Ba	11.8	216.0
^{203}Hg	46.76	279.2
^{51}Cr	27.7	320.0
^{85}Sr	64.85	514.0
^{110m}Ag	252.2	657.2
		937.3
^{134}Cs	752.63	795.8
^{46}Sc	83.8	889.3
		1120.5
^{86}Rb	18.82	1076.6
^{59}Fe	44.56	1099.2
		1291.6
^{65}Zn	244.0	1115.5
^{60}Co	1924.2	1173.2
		1332.5
^{124}Sb	60.2	1691.0

Let us solve this differential equation for the following situation, which is also illustrated in Figure 9.5.

Assuming that Φ, σ and n are constant, this differential equation yields

$$A = \Phi\sigma \frac{wN_A}{M} \%\mathrm{A}(1-\mathrm{e}^{-\lambda t_i})\, \mathrm{e}^{-\lambda t_c} \tag{9.10}$$

where

A = activity, in Bq, dps, at the end of irradiation time of t_i and cooling time of t_c
Φ = flux, n/s cm^2
σ = cross section, cm^2
w = weight of the element in the sample, g
N_A = Avogadro number
M = atomic weight of the element
%A = abundance of the stable isotope A which will produce radioactive isotope of interest
λ = decay constant = 0.693/half-life
t_i = irradiation time
t_c = cooling time

This is the most important equation used in NAA. Equation (9.10) can be further simplified depending on irradiation time and half-life.

We can approximate $(1 - \mathrm{e}^{-\lambda t_i}) = \lambda t_i$, if t_i is small or irradiation time is much smaller than the half-life of the isotope produced. Then $A_0 = \Phi\sigma n\lambda t_i$. If the irradiation time is much longer than the half-life $(1 - \mathrm{e}^{-\lambda t_i}) = 1$ or $A_\infty = \Phi\sigma n$. This is called the saturation activity, or the maximum activity one could get at the end of the irradiation.

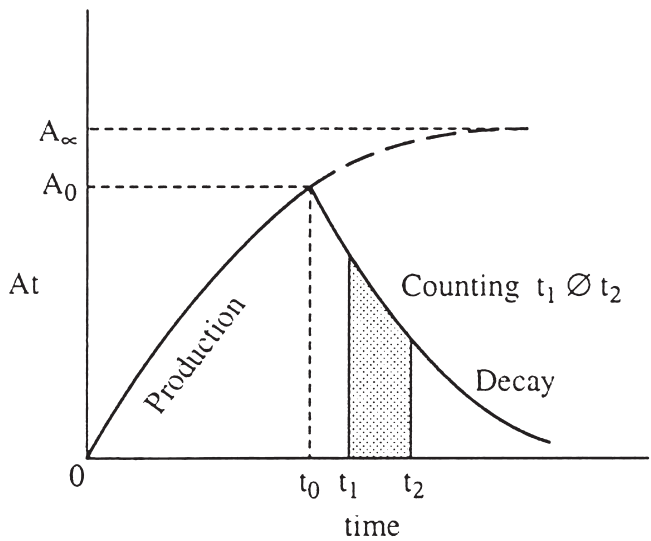


Figure 9.5 *Neutron irradiation and counting*

It is important to take into account the decay of activity during counting period. This is especially necessary if the counting period is equal to or longer than the half-life of the isotope of interest.

Suppose we started counting at time t_1 and ended at t_2. The number of atoms surviving at t_1 and t_2 will be (see Figure 9.5)

$$Nt_1 = N_0 e^{-\lambda t_1} \tag{9.11}$$

$$Nt_2 = N_0 e^{-\lambda t_2} \tag{9.12}$$

If the interference- and background-corrected total peak area is A_p count,

$$A_p = \vee A_0 e^{-\lambda t} = [A_0/1]e^{-\lambda t_1} \quad \text{or} \tag{9.13}$$

$$A_0 = \frac{A_p \lambda e^{\lambda t}}{1 - e^{-\lambda RT}} \tag{9.14}$$

where

A_p = interference and background-corrected total peak area,
A_0 = decay corrected count rate at the end of irradiation,
t_1 = time elapsed between the end of irradiation and the beginning of counting, and
RT = real counting time, $t_2 - t_1$

In activation analysis, quantitative determination can be made by either of the two methods. The direct method, which employs the above equation and the comparative method where a standard is used along with the sample for comparison. In the case of direct method, in principle, all the factors on the right-hand side of the equation are known or can be measured. Thus, it should be possible to calculate the activity. In practice, however, many factors in the above equation may not be known with sufficient accuracy. Therefore, almost in all cases, comparative method is used in NAA, in which both the sample and the standard are irradiated simultaneously, so that the equation can be greatly simplified by using the comparison method, and becomes:

$$w_{samp} = (A_{samp} / A_{std})\, w_{std} \tag{9.15}$$

As seen, one has to know only the activities of interest both in the standard and in the sample, A_{std}, A_{samp}, measured at the same conditions, and the weight of the element in the standard, w_{std}, in order to calculate the weight of the desired element in the unknown sample.

9.3.7 Measurement of Gamma Rays

9.3.7.1 Interaction of Gamma Rays with Matter

The accurate detection of γ- or X-ray radiation is, of course, a basic premise for nuclear activation analysis techniques. Thus, the nature of the interactions of high-energy

(about 0.02–11 000 keV) photons with matter (*e.g.*, the detector material and shielding) must be understood. The cross-section variations with energy of the three possible types of interaction are shown in Figure 9.6. described as follows.

In photoelectric effect, the energy, $h\nu$, of a photon is completely transferred to bound electron, which is ejected from the atom or molecule with an energy

$$E = h\nu - e_b \tag{9.16}$$

where e_b is the binding energy of the electron. If the kinetic energy of the ejected electron were completely absorbed by the surrounding material, *e.g.*, a detector, it would correspond to a *full energy event*. This effect is dominant at energies < 200 keV in germanium.

In Compton effect, the photon transfers only part of its energy to the bound electron and is "Compton-scattered". The electron is ejected from the atom as in the case of the photoelectric effect. This type of interaction gives rise to "partial-energy" events in γ-ray detection systems – the "Compton background" – as well as other

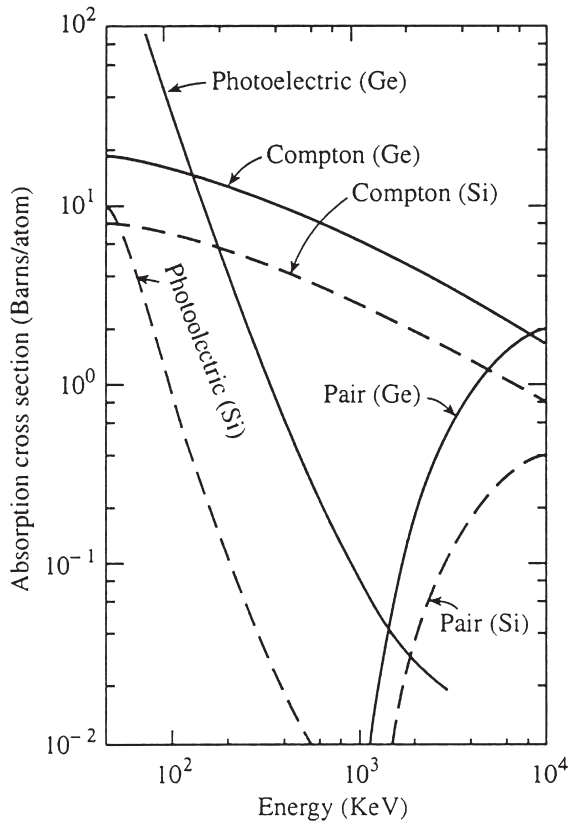


Figure 9.6 *The variation with energy of the photoelectric, Compton, and pair production cross sections in silicon and germanium*

spectral features. The Compton effect is important over the entire energy range, and is the dominant effect from 0.2–8.0 MeV in germanium.

In pair production, a photon with an energy greater than 1.022 MeV can, in the Coulomb field of the nucleus, produce a positron–electron pair plus kinetic energy. The positron, e^+, quickly finds another electron, e^-, in the material's matrix and, immediately, the pair annihilate, yielding two 0.511 MeV γ-rays emitted at essentially 180° angle. Unique spectral features result from this interaction, and are especially important in PGAA. The interaction is important at high energies and dominates above 8 MeV in germanium.

Liquid, plastic, crystalline scintillation and semiconductor detectors are all very important for the measurement of ionizing radiation. But nowadays, hyperpure germanium (HPGe) or intrinsic Ge detectors are commonly used.

Assuming that an incident γ-ray has deposited all of its energy in the active layer of the detector, a small proportional electronic signal is sent to a linear amplifier. The signal is increased proportionally to somewhere in the range of 1–10 V, which is suitable for acceptance by an analog-to-digital converter (ADC). The ADC, in effect, selects a "channel" number proportional to the voltage of the input pulse and assigns one "count" to the address. When many events are recorded and stored in memory, a spectrum is accumulated. This is simply a histogram of counts, proportional to the number of γ-rays emitted from the source – the "intensity" – vs. channel number which is proportional to energy.

The main feature of γ-ray spectrum of ^{60}Co which has energies of 1174 and 1333 keV γ-rays, taken with a HPGe detector is shown in Figure 9.7.

(a) Full energy photopeaks at 1174 and 1333 keV. The γ-rays were completely stopped in the active layer of the detector; none were scattered out.
(b) The Compton "continuum" or "background". These are partial energy events, that is, only partial deposition of energy in the active layer occurred.
(c) Backscatter peak at 0.20–0.25 MeV.
(d) The Compton "edge" is also a result of Compton scattering in the detector itself. For example, if the 1.333 MeV γ-rays is Compton scattered out of the active region of the detector, the minimum energy of the scattered, γ-rays is 0.214 MeV. Therefore, the total energy deposited in the crystal cannot exceed $(1.333 - 0.214) = 1.119$ MeV.
(e) X-ray peaks are produced by the irradiation of the γ-rays from the source with material surrounding or within the detector.
(f) The annihilation peak at 0.511 MeV is a result of pair production and subsequent annihilation of an $e^+ - e^-$ pair in the surrounding or detector material.
(g) Single- and double-escape peaks result from pair production events in the active region of the detector. One or both of the resulting 0.511 MeV γ-rays may escape the detector, resulting in peaks in the spectrum at 0.511 and 1.022 MeV less than the full energy value.

As an example, γ-ray spectrum of a total diet sample is shown in Figure 9.8. This is the spectrum of 250 mg dry total diet sample irradiated for 2 min at NIST reactor.[3] Gamma rays coming from the sample were counted with 150 cc HPGe detector for 500 s. As seen, short half-life isotopes are clearly identified. Figure 9.9 presents the

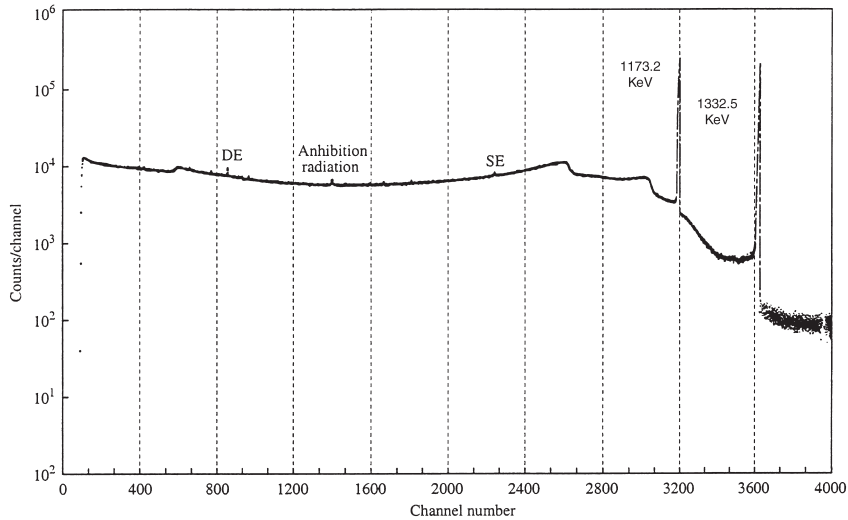


Figure 9.7 *Gamma ray spectrum of ^{60}Co taken with a HPGe detector*

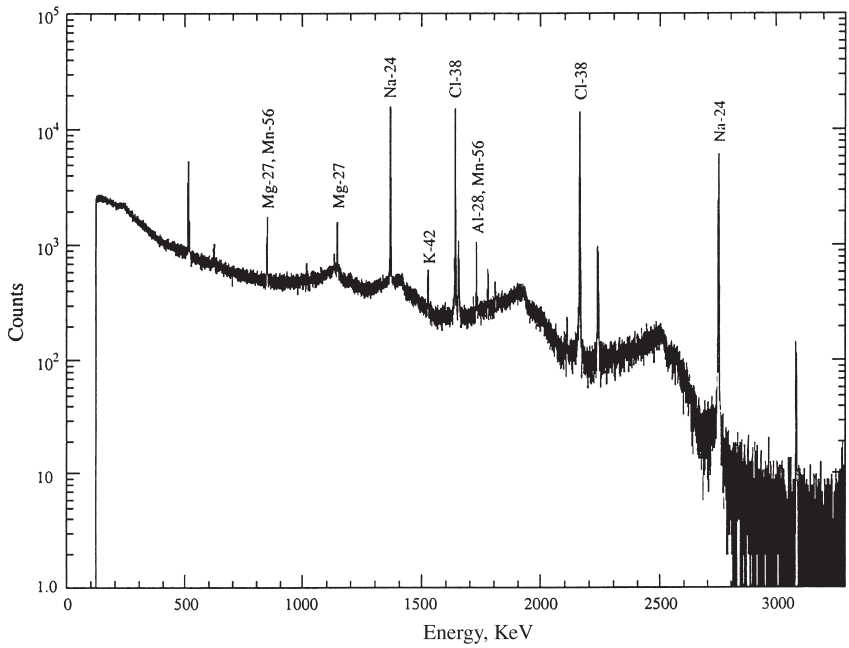


Figure 9.8 *Gamma ray spectrum of total diet sample: result of short irradiation*

γ-ray spectrum of same sample, which was irradiated for 4 h and cooled for few months. This is the long count of a 300 mg dry total diet sample irradiated in a reactor with a neutron flux of 1.2×10^{13} n/s cm^2. As seen, one can identify many peaks corresponding to many isotopes.

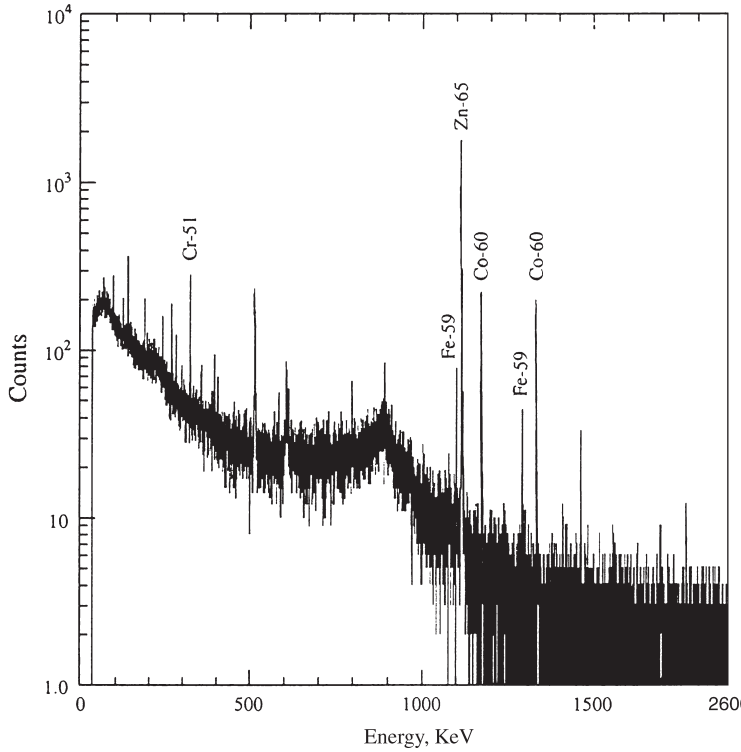


Figure 9.9 *Gamma ray spectrum of total diet sample: result of long irradiation*

9.4 Other Nuclear Techniques

Although INAA is the most used nuclear technique, there are other techniques that can be used for the trace element analysis of food and diet.

Instrumental fast neutron activation analysis (IFNAA) is one of the techniques that is used quite often. Neutron generators through a DT reaction produce the 14 MeV fast neutrons:

$$^{3}H(^{2}H, n)^{4}He$$

The main reactions observed are (n, p), (n, α) and (n, 2n); other reactions are often observed but with lower probability. The advantage of the technique is the measurability of elements such as Si, P and Pb, while Na and Cl do not interfere as they do in INAA.

Neutron capture PGAA is another technique, which is used in number of centres. *Prompt* γ-rays are those emitted in very short time (10^{-12}–10^{-15} s) after formation of the product nucleus in a nuclear reaction. This technique is very useful in measuring the concentration of a number of elements such as H and C which are not suitable to measure with INAA.

9.5 Determination of Trace Elements in Total Diet by Neutron Activation Analysis

Conventional INAA can be used for simultaneous determinations of many elements in food and total diet samples.[4–7] About 100–300 mg of properly prepared dry samples can be subjected to "short runs" by irradiating for 1–10 min, decay for 1–4 min and counting for 10 min for measuring short half-life isotopes of the elements such as Ca, Br, Cl, K, Mg, Mn, Na and I. The same or another set of the samples can be irradiated for 4–10 h, allowing to decay for 2–6 days and counting for 1–6 h for detecting the longer-lived nuclides for As, Co, Cr, Fe, Rb, Sc, Se, Sb, Cs, Hg and Zn determination.

Instrumental neutron activation analysis for trace and ultra-trace elements in a matrix such as total human diet is hampered by the γ-ray radioactivity from ^{24}Na ($t_{1/2}$ = 14.96 h), ^{38}Cl ($t_{1/2}$ = 37.21 min), ^{42}K ($t_{1/2}$ = 12.36 h), and by the Bremsstrahlung from P, a pure β emitter ($t_{1/2}$ = 14.8 d). One may need radiochemical separation to isolate very low radioactivities of the long-lived isotopes, which otherwise cannot be detected in the Compton continuum of highly energetic isotopes and the ^{32}P Bremsstrahlung. It appears that INAA is the method of choice for the elements mentioned above but in general has insufficient sensitivity for Cd, Ni and Sn. Table 9.8 summarizes some representative data obtained by INAA for trace elements in total diet.[3,7]

9.6 Present Status of Activation Analysis by Comparison with Other Analytical Techniques

Activation analysis is best suited for the analysis of solid samples. These can be irradiated without any need for a dissolution step. Of course, liquid samples can also be analysed, but usually preconcentration is then required, for example by freeze-drying or by fixation on an ion-exchange resin.

Sensitivities and detection limits in NAA are determined by several factors, including nuclear constants, experimental parameters and the presence of easily activable elements in the sample. In very favourable cases, detection limits down to the ng kg^{-1} range may be obtained. The sensitivities and detection limit vary in an irregular way from element to element and several elements are difficult or impossible to determine by standard INAA, *e.g.*, Li, Be, B, C, N, O, Si, P, Sn, Tl and Pb. Light elements and elements such as Tl and Pb can, however, be accurately determined by PGAA and photon activation analysis.

A further and extremely important advantage of activation analysis is that contamination can occur only before or during irradiation. Usually the amount of sample handling required before or during the irradiation can be limited. When a post-irradiation radiochemical separation is required, the separation can be carried out without any danger of contamination, and non-radioactive carriers may be added to facilitate the separation.

Activation analysis has some disadvantages, however. For many elements, the turn-around time is long, which may be a serious drawback for routine analysis; the

Table 9.8 *Trace elements in human diet determined by duplicate portion technique using instrumental neutron activation analysis*[3,7]

No	*Sample ID*	*Al (mg kg^{-1})*	*Ca (mg kg^{-1})*	*Cl (mg kg^{-1})*	*Mg (mg kg^{-1})*	*Mn (mg kg^{-1})*	*Na (mg kg^{-1})*
1	SA-69-SM	1.660±0.103	315±12	6163±38	113±6	2.869±0.069	4253±32
2	IO-72-SM	7.772±0.156	391±12	5780±35	101±7	1.858±0.059	4042±30
3	SC-72-SM	2.743±0.150	490±21	6561±43	125±11	3.318±0.093	4466±36
4	TY-74-SM	4.388±0.152	267±13	5803±37	92±8	3.246±0.079	4057±31
5	EG-72-SM	4.223±0.128	828±25	7427±45	183±9	6.796±0.080	4976±36
6	FY-70-SM	4.838±0.168	451±17	5808±37	128±8	5.593±0.083	4409±33
7	AM-74-SM	2.833±0.146	921±22	8512±52	144±7	2.326±0.079	5744±42
8	MA-72-SM	4.236±0.151	672±17	8448±51	86±22	6.106±0.091	5806±42
9	AA-71-SM	2.999±0.093	395±11	3650±23	129±5	3.830±0.056	2462±19
10	YS-69-SM	1.875±0.094	412±24	7055±50	135±17	3.699±0.031	5289±10
11	ILO-70-SM	3.858±0.171	314±14	5162±34	108±10	3.887±0.078	3532±28
12	SG-75-SM	2.828±0.112	538±16	5975±37	185±9	3.020±0.073	4359±32
13	RO-72-SM	6.356±0.155	303±11	6466±40	143±7	4.943±0.077	4500±33
14	MD-69-SM	3.804±0.135	522±18	9169±55	241±11	8.988±0.105	6590±47
15	AN-69-SM	5.321±0.139	295±12	6018±16	112±13	2.567±0.068	4118±31
16	HA-72-SF	5.364±0.169	315±16	7105±44	131±10	4.546±0.084	5089±38
17	YC-72-SF	8.213±0.193	532±18	6485±15	162±10	4.549±0.086	4445±33
18	FTC-72-SF	20.281±0.325	244±13	5156±33	162±8	6.916±0.082	3700±28
19	NS-76-SF	2.450±0.373	525±14	6822±42	142±12	4.189±0.076	4659±34
20	SEA-68-SF	1.947±0.109	543±14	5467±34	127±8	2.177±0.062	3758±28
21	KT-77-SF	2.586±0.134	387±13	5438±34	166±8	2.909±0.062	3725±28
22	SO-80-SF	1.683±0.096	325±13	6445±40	135±9	2.001±0.063	4467±33
23	YU-78-SF	1.727±0.126	801±27	1.771±0.010[1]	⩽41	⩽1.9	1.237±0.009[1]
24	SS-76-SF	2.786±0.105	813±19	7580±46	153±11	3.210±0.077	5695±41
25	NY-80-SF	1.905±0.140	490±16	7788±48	233±10	4.120±0.081	5319±39
26	NG-79-SF	5.323±0.191	381±15	6914±43	215±11	7.494±0.098	4867±37
27	CS-79-SF	3.599±0.134	234±12	5618±35	144±7	4.080±0.067	4002±30
28	EB-77-SF	5.373±0.140	716±18	7439±45	155±8	3.304±0.068	5079±37
29	KFA-69-SF	2.934±0.134	635±24	6375±40	140±9	2.213±0.069	4404±33
30	SZP-76-SF	2.886±0.132	830±18	6180±38	134±8	2.234±0.065	4253±32

Experimental condition: 30 s. Irradiation at NIST RT-4 reactor. First count: ~20 cm for 5 min after 2–3 min decay. Second count: ~20 cm for 5 min after 10 min decay. SM: Summer Male; SF: Summer Female samples.

method may be labour intensive, but this can be reduced by automation; a nuclear reactor or other source of activating particles is required.

When compared with other sensitive analytical multi-element methods often used for the same purpose as activation analysis, such as ICP MS, NAA has the following advantages:

(1) For solid samples, NAA has the definite advantage of not requiring sample dissolution, with the problems of difficult or incomplete dissolution of some matrices, possible losses of some elements such as As, Se or Hg by volatilization, or precipitation or adsorption losses. In addition, no blank is introduced from acids or other reagents, which is a common major source of contamination. The latter advantage holds also for radiochemical NAA. This advantage is very important for materials with low analyte levels that require considerable effort for contamination-free dissolution, *e.g.*, semiconductors and biological samples.

(2) Probably the most important advantage of activation analysis is that, whenever interference occur, for instance in the analysis food and diet samples, where the gamma radioactivity of ^{24}Na, ^{38}Cl, ^{42}K and Bremsstrahlung from ^{32}P are the main matrix activities, the radionuclide of interest can be carried through even complex radiochemical separations without the danger of contamination and with addition of non-radioactive carriers to ease the separation.

(3) In addition, since the activation analysis is based on a principle quite different as compared to other spectroscopic techniques, its use is often an important asset in studies regarding to analysis of standard reference materials providing a different approach.

References

1. G. Hevesy, H. Levi and Kgl. Danske Videnskab, *Selskab. Math. Fys. Med.*, 1936, **14**(5).
2. A. Chatt, H.S. Dang, B.B. Fong, J. McDowell and D.L. Pegg, *J. Radioanal. Nucl. Chem.*, 1988, **124**, 65.
3. R. Lindstrum, private com. 1999.
4. S.M. Liu, C. Chung, J.T. Chuang, C.F. Wang and N.K. Aras, *J. Radioanal. Nucl. Chem.*, 1991, **150**, 397.
5. T. Mumcu, I. Gokmen, A. Gokmen, R. Parr and N.K. Aras, *J. Radioanal. Nucl. Chem.*, 1988, **24**, 289.
6. D.L. Samudralwar and A.N. Garg, *Fresenius J. Anal. Chem.*, 1994, **348**, 433.
7. N.K. Aras and I. Olmez, *Nutrition* (Supplement), 1995, **11**, 506.

CHAPTER 10

X-Ray Methods

10.1 Introduction

X-rays are electromagnetic radiation with a wavelength between 0.001 and 10 nm. They are produced when high-energy electrons decelerate or when electron transitions occur in the inner shells of atoms. Conventional X-ray spectroscopy is largely confined to the region from 0.01 nm (UK_α to 2 nm (FK_α) and the energy and wavelength of X-ray photons are related by

$$E\ (\text{keV}) = 1.24\ \lambda^{-1} \tag{10.1}$$

where UK_α and FK_α are the K_α X-rays of uranium and fluorine, respectively, and λ is the wavelength in nm.

The emission of characteristic X-rays following atomic excitation has long been used for elemental analysis. The variety of new systems that are becoming available and the number of books, reviews and general articles, which appear in the literature, indicate the increasing interest in X-ray methods, especially for environmental, biological and food studies.[1–3]

Equipment with a cathode tube as primary X-ray source and a detection system consisting of a diffraction spectrometer and a proportional counter has long been available for X-ray fluorescence analysis. The development of semiconductor X-ray detectors and the availability of particle accelerators have made possible the development of many new techniques and applications. Nowadays, protons and other heavy particles are used very effectively for the excitation of the atoms since they provide very high cross-sections for the production of K and L X-rays. The detection of the X-rays is much more efficient with a semiconductor detector than with diffraction spectrometer and the possibilities of automation are also improved. Owing to these factors, it has been possible to develop particle-induced X-ray emission (PIXE) analysis into a method having the advantages of high sensitivity, speed and automation for simultaneous multi-element determinations. X-ray methods are applicable to practically all elements up to uranium, although there are some difficulties for the light elements.

10.2 Basic Principles

The interaction of X-rays or charged particles with the electron cloud of an atom creates electron holes, leaving the atom in an excited state. A large number of K and L holes are produced only with high-energy X-rays and charged particles. The lifetime of K and L holes varies between 10^{-9} and 10^{-16} s. The holes may be filled by any electron with higher energy, and the extra energy is either emitted as a X-ray or as an Auger electron. The principle of all the X-ray methods for trace element analysis is based on the determination of this secondary or characteristic X-rays emitted.

The ratio of the X-rays emitted divided by the total number of holes is defined as the fluorescence yield for each of the shells. The different X-ray groups are labelled by capital letters corresponding to the shells with the electron vacancies, and suffixes for the electrons which drop into the vacancies (K_α, K_β, L_α, *etc.*). The number of these X-ray groups with different energies is very large, corresponding to the various combinations of atomic electron shells and subshells. However, when one detects the characteristic X-rays in the range of 1 to 25 keV, a relatively small number of high-intensity peaks are observed corresponding to the transitions shown in Figure 10.1.

The energy of each of the X-ray lines is characteristic of the elements, and increases with increasing atomic number (Figure 10.2).[1] This is an important feature allowing the identification of the atom from which an X-ray with specific energy has been emitted.

The differences in energy between the levels increase gradually with the atomic number, so that the radiation for the K series has higher energy for the heavier

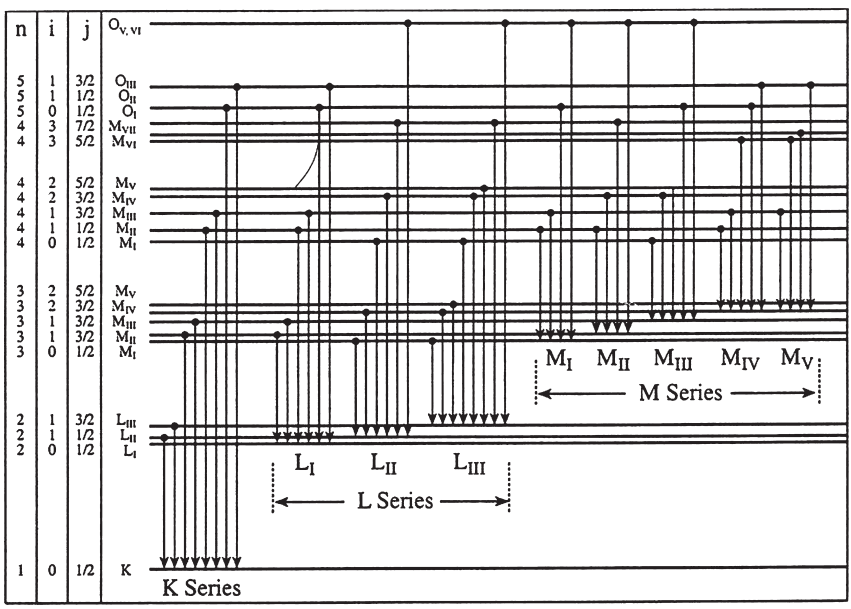


Figure 10. 1 *Atomic energy level diagram for main K and L X-ray transitions*[1]

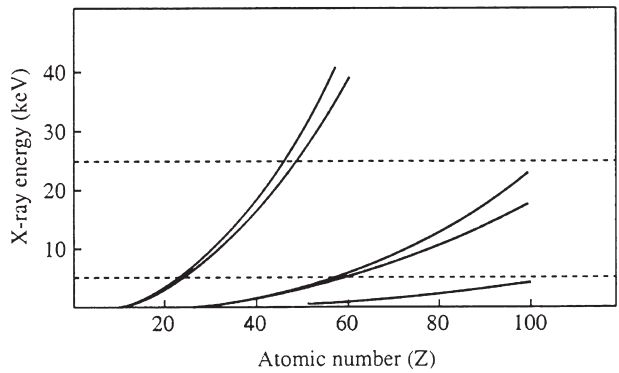


Figure 10.2 *Different X-ray energies as a function of atomic number*[1]

elements (Figure 10. 2). Moseley's law describes the frequency of the characteristic X-rays within any series (*e.g.* K or L):

$$\nu = k(Z - 1)^2 \tag{10.2}$$

where ν is the frequency of the X-ray, Z the atomic number of the target element and k a constant.

10.3 X-Ray Fluorescence Spectrometry

As X-rays pass through matter, they are attenuated as a consequence of absorption and scattering by an amount depending upon the thickness and the density of the absorber. X-rays of different wavelengths are attenuated to a different extent. The effect of scattering is small for all but the lightest elements, and it can be neglected in wavelength regions where appreciable absorption occurs.

The absorption spectrum of an element is simple and consists of a few well-defined peaks with a wavelength characteristic of the element and largely independent of its chemical form. Figure 10.3 shows the absorption spectrum for tungsten. In the spectrum, there are sharp discontinuities called absorption edges.

It appears that there is one K edge and three L edges. At the K edge, the energy of the photon exactly matches the energy needed to eject the K electron from the element, so that here the probability for absorption is the highest. Immediately above this wavelength, the energy is insufficient to remove a K electron.

Beer's law describes the absorption of X-ray radiation as

$$\ln(I_0/I) = 2.303 \log (I_0/I) = \mu x \tag{10.3}$$

where x is the sample thickness in meter, I and I_0 are the intensities of the transmitted and the incident beam through a sample of thickness x and μ is the *linear absorption coefficient*. The numerical value of μ depends on the nature of the element and

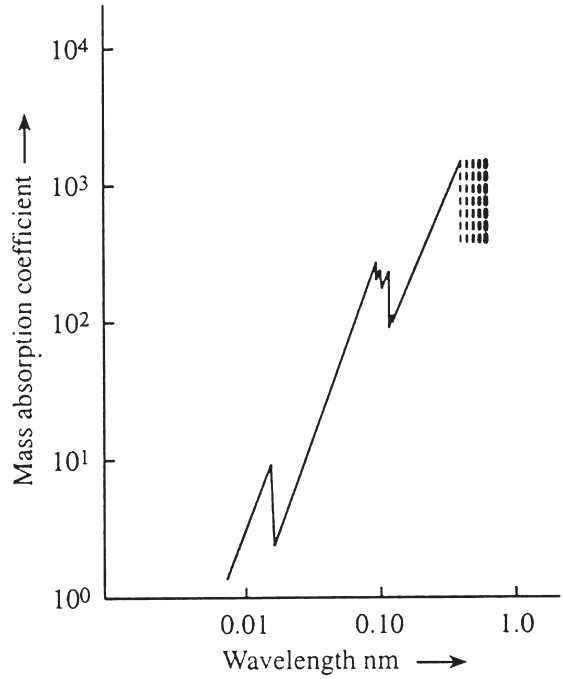


Figure 10.3 *Mass absorption coefficient of tungsten as a function of wavelength*

the number of its atoms per cross-sectional area through which the beam is travelling. It is customary to express the coefficient as a *mass absorption coefficient*, μ_m:

$$\mu_m = \mu_\rho \tag{10.4}$$

where ρ is the density of the absorbing substance. Equation (10.4) indicates that an exponential absorption law governs X-ray absorption. The mass absorption coefficient is expressed in $m^2\ kg^{-1}$ and is independent of the physical state of the element.

10.3.1 Production of X-Rays

Two main excitation techniques are used in X-ray fluorescence spectrometry (XRF): (a) X-ray tube excitation and (b) radioisotope excitation.

X-ray tube excitation: A conventional X-ray tube is an evacuated tube in which a tungsten filament cathode and an anode are mounted (Figure 10.4). The filament is heated by means of a current producing a region of high electron density around the cathode. Electrons impinging on the anode produce X-rays, a significant portion of which passes through the window. The choice of anode material and the window characteristics can be critical. Any solid material, except copper, molybdenum and tungsten are the most frequently used for general purposes. The emission spectrum of molybdenum as used as an anode is shown in Figure 10.5, in which K_α and K_β lines are identified.

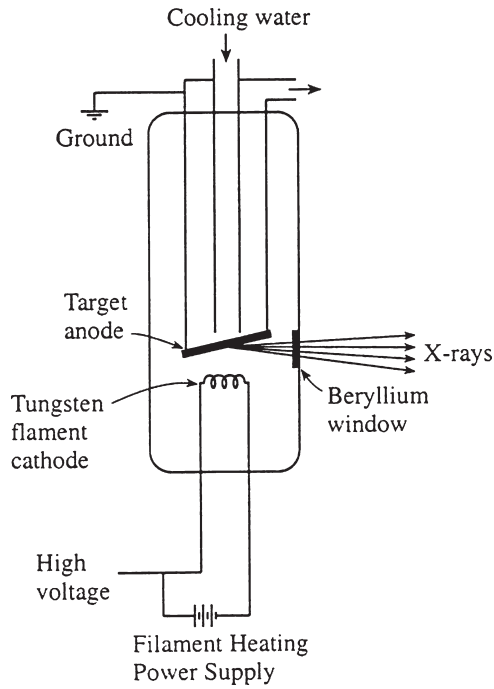


Figure 10.4 *Sealed X-Ray tube*

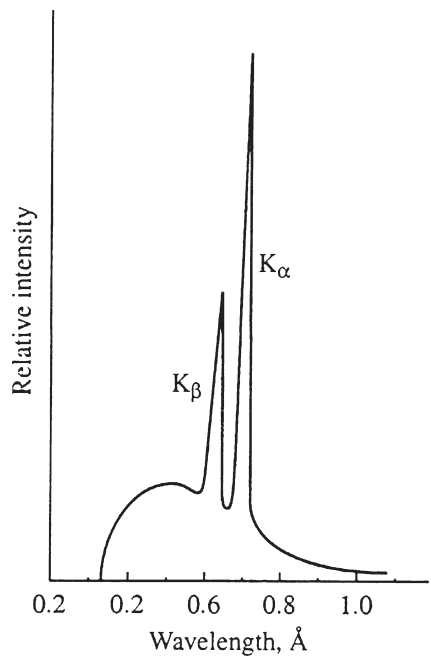


Figure 10.5 *Line spectrum of molybdenum*

(b) *Radioisotope excitation*: Instead of an X-ray tube, a radioactive source such as ^{109}Cd, ^{241}Am, *etc*. may be used to excite the target sample, utilizing either γ- or X-rays. Radioactive sources are widely available in small, encapsulated and easily handled source, which emits γ- or X-rays at a variety of energies. Table 10.1 shows common radioisotope sources and their properties used in XRF. For radiation protection purposes and other reasons, the intensity of the source is usually limited to 5–10 mCi. The beam intensity produced by radioactive sources is low compared with the beam from a cathode-ray tube. Therefore, although radioactive sources are practical and low cost, they provide a limited range and lower sensitivity as compared to X-ray tube excitation in trace element determination.

10.3.2 Wavelength Dispersive X-Ray Fluorescence Spectrometry

Any type of XRF unit must consist of a primary beam source, preferably a cathode tube with variable X-ray energy, or a suitable radioactive source, and the sample, which can be in vacuum, in helium, or in air. The X-rays emitted by the sample must be converted into a spectrum so that the characteristic X-rays of each element can be measured. The detection and analysis system of the secondary X-rays consists of a diffraction spectrometer with a crystal, a proportional counter and a mechanism for changing the diffraction angle. In wavelength dispersive X-ray fluorescence spectrometry (WDXRF), which is the older technique, the X-rays emitted by the sample are dispersed spatially on the basis of their wavelengths by crystal diffraction before detection. The characteristic X-rays are dispersed (Figure 10.6) by an analysing crystal or pseudocrystal on the basis of Bragg's law, which states that diffraction occurs on the condition that

$$N_\lambda = 2d \sin \theta \tag{10.5}$$

where n is an integer, λ the wavelength considered, d the interlattice spacing of the crystal and θ the angle of incidence and of emergence of the radiation considered.

Crystalline materials with inter-atomic distances of 0.14–1.32 nm are usually used to disperse the X-rays from the sample into a wavelength spectrum. Various crystals are available, and usually one with d values such that a θ in the range 20–80° is selected. Most commonly used are lithium fluoride, pentaerythritol, ammonium dihydrogen phosphate (ADP) and potassium hydrogen phthalate. Recently developed

Table 10.1 *Common radioisotope sources used in XRF*

Nuclide	*Half-Life*	*Type of Radiation*	*Photon Energies (keV)*	*Element Excited*
^{55}Fe	2.7 years	Mn, K X-rays	5.9	V and below
^{109}Cd	453 days	Ag, K X-rays	22.1	Cu–Mo (K shell)
^{153}Gd	242 days	Eu, K X-rays	41	Mo–Ce (K shell)
^{241}Am	458 years	γ-ray and Np L X-rays	59.6 γ-ray and Np L X-rays	Sn–Tm (K shell)
^{57}Co	267 days		14,122,136	Ta–U (K shell)

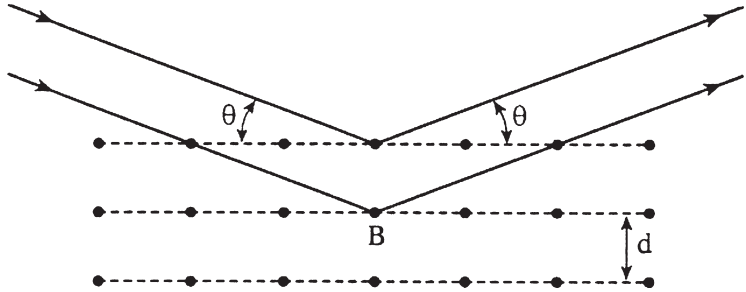


Figure 10.6 *Diffraction of an X-ray by a crystal*

synthetic pseudocrystals with *d* spacing of 2.2–8.0 nm are very efficient for the X-rays from light elements (B to Mg). Figure 10.7 gives an experimental arrangement for WDXRF. Excitation is done, in this case, by an X-ray tube. A recording system is also required to record the intensity of the analysed beam as a function of the diffraction angle, or the X-ray energy.

Scaled gas proportional counters are used to detect intermediate energy X-rays, *e.g*. Cl K_α through Ni K_α. Gas flow proportional detectors are used to detect low-energy X-rays, *e.g*. B K_α through S K_α. Scintillation detectors are used for high X-ray energy, above Ni K_α.

Such WDXRF systems are fairly simple in operation and they have good energy resolution, *i.e*. good separation of the various characteristic peaks. On the other hand, they are quite slow, requiring long counting times, and the detection sensitivity is not very good and is limited to a narrow range of atomic numbers depending on the energy of the primary beam.

10.3.3 Energy Dispersive X-Ray Fluorescence

In energy dispersive X-ray fluorescence (EDXRF), the X-rays from the sample are measured with a Si(Li) or a high-purity germanium detector, HPGe, which produces pulses proportional to the energy of the impinging X-rays and is connected to a multi-channel analyser similar to those used for γ-ray spectrometry, as discussed in Chapter 9. X-rays of all energies are measured simultaneously, so that EDXRF is truly a simultaneous multi-element technique.

The schematic diagram of an EDXRF system with a radioisotope excitation is shown in Figure 10.8. The system is simpler and more compact than the tube system, the energy resolution is poorer, and the detection sensitivity is lower.

The excellent resolution provided by the Si(Li) or HPGe detectors has been a significant breakthrough in X-ray analysis. The resolution of an X-ray detector is usually defined as the full-width at half-maximum (FWHM) of a spectral peak and is typically less than 145 eV at 5.9 keV for a Si(Li) detector. To obtain optimum performance with these semiconductor detectors, low-noise preamplifiers and amplifiers are required along with cooling in liquid nitrogen of the detector. The HPGe detector is used when X-rays above 30 keV are produced, as it is more efficient than the Si(Li) detector in this energy range. Its resolution is about 180 eV at 5.9 keV and

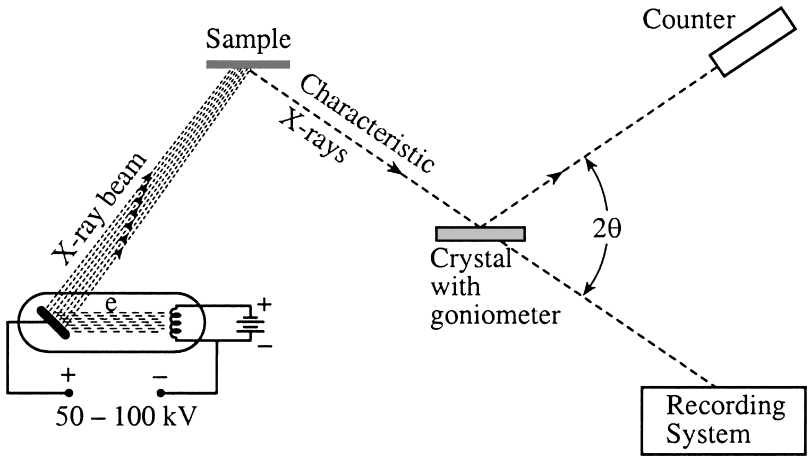


Figure 10.7 *Wavelength dispersive X-ray fluorescence system*

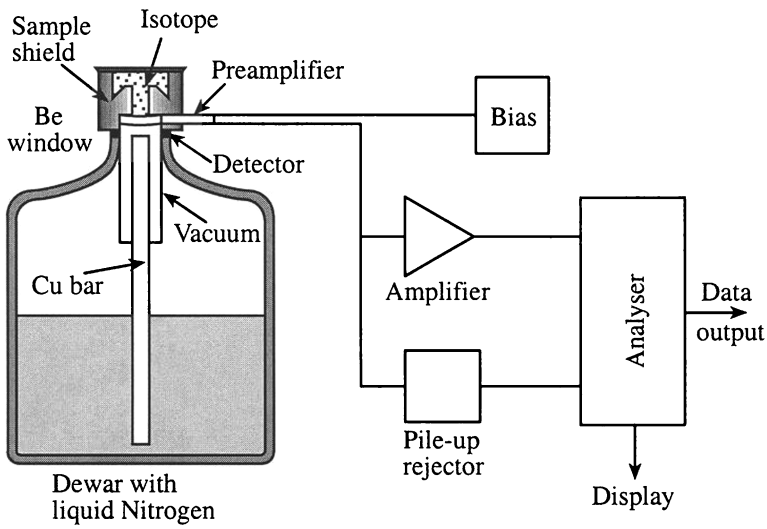


Figure 10.8 *Energy dispersive X-ray fluorescence system with a radioisotope source and a HPGe detector*

400 eV at 122 keV. With such a detector, the K lines of high-Z elements can be measured, rather than the L lines, which would have suffered interference by K lines of low-Z elements.

10.3.4 Total Reflection X-Ray Fluorescence Spectrometry

In total reflection X-ray fluorescence (TXRF), which is in fact a variant of EDXRF, the sample material is prepared as a thin film on a polished, optically flat carrier.[5,6]

A collimated beam from the X-ray tube impinges on the optically flat surface of the sample carrier at an angle of incidence below the critical angle and is thus totally reflected. Total reflection can occur if radiation goes from an optically dense medium to one that is optically less dense. As for X-rays, any medium is optically thinner than a vacuum; total reflection is possible on condition that the angle of incidence is less than the critical angle, which depends on the properties of the carrier and the photon energy.[5] For photon energies of ~10 keV, the critical angle is about 0.1°. The excited sample emits fluorescence radiation. The emitted X-rays, which is doubled in intensity because of the sample by both incident and reflected beams, are detected by a Si(Li) detector placed immediately above the sample and are further processed as in EDXRF. The most common carrier materials are quartz, Plexi glass, glassy carbon and boron nitride.

The depth of penetration of the X-ray beam depends on the angle of incidence; at large angles it will penetrate several mm to several hundreds of mm, but in the case of total reflection it penetrates only 3 nm. The carrier thus corresponds to an extremely thin foil, so that the incident radiation has virtually no interaction with the carrier, resulting in a very low scattering intensity. As a consequence, the background under the peaks in the fluorescence spectrum is substantially reduced and detection limits are improved. In addition, however, the sample must be in the form of a very thin film or a few very fine grains in order not to disturb total reflection at the carrier. Also, the high-energy Bremsstrahlung part must be removed from the spectrum of the beam from the X-ray tube, as this would not be totally reflected but would penetrate deeply into the carrier, thus contributing to scatter and spectral background. A single- or double-quartz reflector placed between the X-ray tube and the sample may serve as an efficient filter.

The low background intensity in TXRF results in substantially better detection limits than in conventional EDXRF. For a large number of elements, absolute amounts between 2 and l0 pg is detectable with a 1000 s counting time. Since the samples need to be in the form of a thin film, matrix effects do not play any significant role and quantification is quite straightforward. Usually a single element, like cobalt, not present in the sample is added as an internal standard and a calibration curve is established which is valid for all matrices.

Recently, portable TXRF spectrometers were bulit for field investigation.[7,8] The X-ray source is a low-power metal ceramic tube with a Mo anode, operating at 40 keV and 1.0 mA. This low rating makes it possible to use a simple air-cooling. The small portable generator produces the necessary energy for the equipment.[9] The monochromator is a Ni/C multilayer. With this excitation, the elements from Si to Zr (K series) and Rh to U (L series) can be determined. The most important difference compared to stationary TXRF spectrometers is the detector. All standard detectors require cooling with liquid nitrogen, so they cannot be used in the field. The new X-flash detector in the PicoTAX requires only Peltier cooling; therefore, the equipment can be transported and used in the field without special requirements. In comparison to the stationary equipments, greater care must be taken during the sample preparation on the carrier plate, because of the detection area of only 10 mm^2. The XRF spectrum of natural water taken with such a system[7] is given in Figure 10.9.

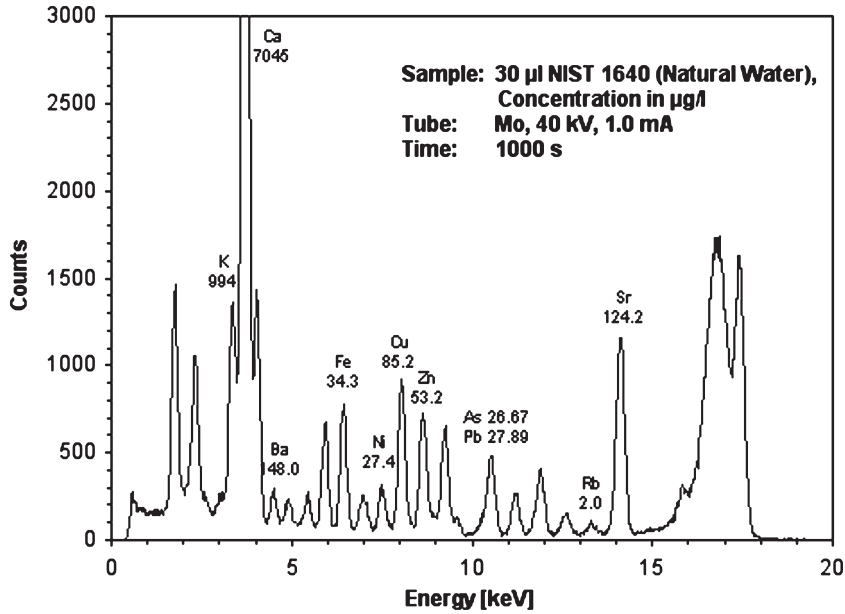


Figure 10.9 *X-ray spectrum of natural water taken with portable TXRF*

10.4 Particle-Induced X-Ray Emission Spectrometry

In PIXE analysis, a beam of heavy, charged particles is used to irradiate the sample. The charged particles eject inner shell electrons from the atoms. The resulting vacancies are filled with electrons from an outer shell, whereby characteristic X-rays are emitted exactly as in XRF.

PIXE was introduced by Johansson *et al.*[4] in 1970. Protons, α particles and even heavy ions have all been used as projectiles, but for most applications, protons with energy between 2 and 4 MeV are preferred. These projectiles are generally obtained from a small particle accelerator. The most popular one is a single-ended Van de Graaff accelerator, in which a continuously moving belt transports charge to a terminal to keep it at a potential of 2–3 MeV in a tank under high pressure. Acceleration of particles from this potential to the earth imparts energies of 2–3 MeV to protons and 4–6 MeV to doubly charged helium ions. These machines yield quite high currents, up to several hundred mA, but this is not a limiting factor.

More recently, small tandem accelerators have been developed for applications such as PIXE and are commercially available. These machines require only half the voltage of a conventional machine, which results in a reduction in size and cost. Compact cyclotrons are also often used for PIXE. It is clear that such expensive accelerators are usually not specifically installed for PIXE analysis, but once installed for other applications, it is easy to include X-ray equipment in the experimental set-up. Of course, as one needs to have access to particle accelerator for PIXE, the technique is less widespread than XRF.

Figure 10.10 shows an experimental set-up used to irradiate samples in vacuum. Irradiation in vacuum is most often applied as it allows even low-Z elements, down to sodium, to be determined and provides better detection limits than irradiation at atmospheric pressure. After proper collimating, the beam enters the target chamber and impinges on the sample. In order to avoid frequent opening and revacuming of this chamber, a sample handling system is provided. A large number of samples and standards can be kept under vacuum and in turn placed in the beam by a sample changing mechanism. The sample itself is typically a thin, pure backing foil (Mylar or polycarbonate) on which a film of biological material, food, an amount of aerosol, or the residue from dried drops of liquid is deposited.

The charged particle beam passes through such thin samples, each particle losing a part of its energy, and can be monitored by a Faraday cup. For the detection of the X-rays from the sample, as in the case of EDXRF, a Si(Li) or a HPGe detector can be used. Such a detector combines high efficiency in the X-ray energy region of interest (2–20 keV) with good energy resolution.

10.5 Quantitative Determination in XRF Methods

The basis of quantitation for all X-ray techniques are that there exists a relationship between the net peak area of an X-ray line in the spectrum and the amount of element in the sample. One of the two methods can be applied for calibration:

(a) The calibration is done by means of thin-film standards, *i.e. the relative method.*
(b) One can use fundamental physical parameters in combination with an experimentally determined efficiency curve, giving the detection efficiency of the detector used as a function of energy; this is the *absolute method*. In order to determine the net peak areas, least-squares fitting routines as in EDXRF are used.

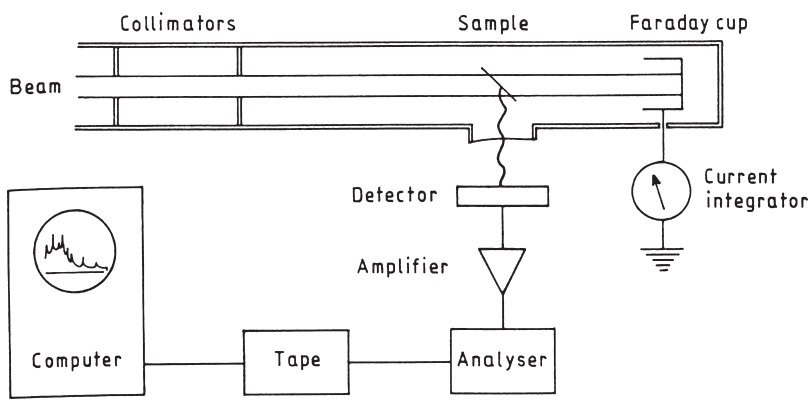


Figure10.10 *Set-up for PIXE*

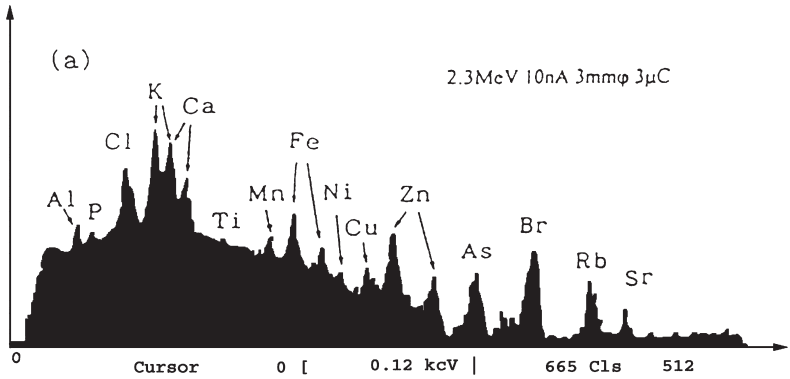


Figure 10.11 *A PIXE spectrum of a 5 mg total diet sample.*[3] *The spectrum is obtained with a Si(Li) detector. Irradiation time was 666 s*

In addition, a correction for matrix effect is usually required, as in general, matrix effects are not negligible. As the physics of these matrix effects is very well understood, their magnitude can in general be estimated *a priori.* This usually allows samples to be so prepared that the matrix effect is not too large. In the case of PIXE, one must take into account the energy loss of the charged particles on their way through the target, and the attenuation of emerging X-rays by the sample. Unlike X-rays, charged particles lose energy with depth in the target, but the number of particles in the beam remains the same until eventually all the particles are stopped.

Application of XRF and PIXE techniques in the biomedical and environmental fields, in geoscience, art and archeology and forensic science, together with industrial applications, have all been described. An excellent overview of the first three areas of application is given in Ref. 5.

The PIXE technique can be applied to measurements of trace elements in food and total diet samples. Figure 10.11 shows a typical PIXE spectrum of a total diet sample.[3] About 5 mg of diet sample was formed into a neat tablet of 5 mm diameter and 0.7 mm thickness. It was irradiated for about 5 min with a 2.3 MeV proton beam from 3 MeV Van de Graaf accelerator in a vacuum at a rate of 10 nA up to 3 mC of the integrated beam current.

Although the measurement part of the technique is exactly the same as in EDXRF, the different method of excitation makes EDXRF and PIXE spectra (as in Figure 10.11) slightly different in appearance. In XRF, the creation of a vacancy in an inner shell is most efficient for energy of the exciting photon just above the binding energy of the electron. The intensity of the characteristic lines therefore decreases with decreasing atomic number, also reflecting the decreasing fluorescence yield. In PIXE, for the energies generally used, ionization cross-sections, a measure of the probability of ionization, are highest for light elements and decrease with increasing atomic number. Therefore the peaks of the lighter elements are much larger than of the heavy elements.

References

1. V. Valcovic, *Trace Element Analysis*, Taylor and Francis, UK, 1987.
2. W. Maenhaut, *Anal. Chem. Acta*, 1987, **195**, 125.
3. M. Yukawa, Y. Watanabe, Y. Nishimura, K. Shiraishi and H. Kawamura, *Proceedings of International IAEA Symposium on Harmonization of Health Related Environmental Measurements Using Nuclear Techniques*, Hyderabad, India, 1996.
4. S.A.E. Johansson and J.L. Campbell, *PIXE: A Novel Technique for Elemental Analysis*, Wiley, Chichester, 1988.
5. A. Pranga and H. Schwenke, Trace element analysis using total reflection X-ray fluoresence spectrometry, *Adv. X-ray Anal*., 35, **899**, 1992.
6. R. Klockenkamper, J. Knoth, A. Pranga and H. Schwenke, Total reflection X-ray fluorescence spectroscopy, *Anal. Chem*., 64, **1115A**, 1992.
7. Pico TAX, Roentec., Berlin, Germany, 2000.
8. M. Mages, S. Woelfl, M. Ovari and W.V. Tümpling jun, *Spectrochim. Acta, B*, 2003, **58**, 2219.
9. R.E.A. Jimenez, *Spectrochim. Acta*, *B*, 2001, **56**, 2331.

CHAPTER 11

Speciation Analysis

11.1 Importance of Speciation Analysis and Related Terms

Essentially, most of the analytical techniques, such as X-ray methods, nuclear activation analysis and atomic spectrometry are selective to an element, since it is characteristics of the atomic or nuclear structure that induces the analytical signal. In the last 40 years, significant advances in instrumental analysis techniques made feasible the elemental determinations at pg level, a routine procedure in chemistry laboratories. The information on *total element concentration*, therefore, became easily available in research related to food and diet as well as the other areas where chemical analysis is employed. Although the total element concentration is still an extremely useful information, the concentration of the analyte element in its different forms, or *species*, has become increasingly important.

For many analytical chemists, the first alarm on the importance of the speciation analysis originated with the Minamata incident, which took place in Japan in the mid-1950s; more than 50 casualties were recorded among the people who were feeding on fish containing methyl mercury.[1] Some other incidents that caused further awareness on the importance of speciation analysis were, tetraalkyllead spillage in Mediterranean as a result of a sea accident[2] and the severe decrease in oyster population in Southern France possibly caused by butyltin pollution from antifouling paints.[3]

There are many chemical and physical procedures exist in order to determine species of an element. The effort by a large number of scientists naturally has caused some confusion and different uses of the terms in this field. Although many scientists still use the terms on speciation in different ways, a recent article that has been published as an *IUPAC recommendation* is extremely useful and important for the clarification of some terms.[4] This work is the product of a combined study by several commissions on trace analysis, environmental chemistry and toxicology. According to this chapter, *speciation* is the distribution of an element among defined chemical species. On the other hand, *speciation analysis* and *fractionation* refer to analytical activities for speciation.

Speciation analysis is conducted in the following structural aspect:[4]

(i) *Isotopic composition*. Natural abundance distribution of isotopes for an element may change by effects of nuclear activities, experiments and accidents.

It is often required to carry out a speciation analysis in order to assess the distribution of an element among its measurable isotopic forms.

(ii) *Electronic and oxidation states*. This property is also called *valency.* Oxidation state of an element is often important in many cases of speciation analysis. For example, it is well known that 3+ state of As, AsO_3^{3-}, and 6+ state of Cr, CrO_4^{2-}, are significantly more toxic than 5+ state, AsO_4^{3-}, and 3+ state, Cr^{3+}, respectively.

A molecule containing a metal of interest possesses all the molecular characteristics of the species not only due to that metal, but also to the rest of the molecule. Therefore, in many cases, the chemical and physical characteristics, such as size, polarity, solubility, adsorption and bonding ability of the molecule determines its interactions in biological systems with consequences related to essentiality, bioavailability and toxicity. It has been suggested that while the relatively small molecules are more important in toxicity, the larger molecules have higher bioavailabilities.[5] The groups given below (iii–vi) are related to molecular speciation

(iii) *Inorganic compounds and complexes.* Elements are often in a compound or in a complex form with inorganic ligands such as chloride, sulfide, oxide or hydroxide. Hydroxo forms are the most commonly occurring species.

(iv) *Organic complexes*. Some organic metal chelates are very stable, such as ferrioxamine, while many others can rapidly change their forms during analysis. Therefore, especially this type of speciation analysis requires considering kinetic as well as thermodynamic effects.

(v) *Organometallic compounds*. These are the molecules where a metal is bound to a carbon atom through a covalent bonding. Products of methylation, alkylation and phenylation are among the members of this group. In general, organometallic forms are more toxic than the inorganic ones; methylation of mercury is an example. On the other hand, for some elements, such as As and Se, methylation produces non-toxic species.

(vi) *Macromolecular compounds and complexes.* This is considered as the highest structural level regarding speciation. In this group, elements are bound to large molecules such as proteins or polyanions such as humic acid. The well-known Fe(III) carrier transferrin is in this group.

Fractionation is another analytical approach for determining the distribution of elements in different forms. It is usually on the basis of size, solubility, affinity and hydrophobicity. For example, in a soil sample carbonates of Ca, Mg and Fe will easily dissolve in an acidic solution but the same metals in a silicate or other refractory compound will not be leached at any pH. In some samples, fractionation related to the physical state of analyte may be of concern. For example, in Hg related air pollution, the concentrations of free mercury vapour (Hg^0), mercury contained in solid particulate material in air and in aqueous media (rain and snow) supply different data regarding the nature of pollution. In food products, such a classification will involve the gas–liquid phases in a carbonated beverage and liquid–solid phases in milk.

As seen, speciation analysis is directly related to food and diet research. The quantitative information obtained from the speciation analysis is significant in many occasions as discussed below.

Speciation is important in *toxicological* studies. Environmental pollution caused by several industrial processes as well as the fertilizers, insecticides, pesticides and herbicides directly used in food production have made the toxicological analysis of these products an absolute necessity. The outputs of these studies do not only bring awareness to the consumer, but also trigger the novel research in toxicology and legislation regarding the maximum levels of toxic materials allowed in the food products. Therefore, speciation analysis has direct significance for human life.

The research on toxicological speciation analysis has not always brought a different look at the existence of new threats to human health. In some occasions, a detailed speciation analysis may prove that the present toxicity is actually lower than the value suggested by the total element concentration, if it is shown that the majority of the species have low or no toxicity. The well-known example is the As content in foodstuffs, in particular sea products, since now it is known that a large majority of this element is present as the non-toxic arsenobetaine. Similarly, arsenocholine and arseno-sugars are non-toxic.

Another field where the speciation analysis is important is the studies related to the *bioavailability* of elements. The metals as well as the vitamins are suggested as supplements for better health. However, a critical balance exists in the body, especially for the elements having a narrow range between the values of essentiality and toxicity; Fe and Se are typical examples. The speciation analysis is also important for food preservation; free copper ions (Cu^{2+}) have been found to be responsible for deterioration of milk, oils and fats. The relation between the speciation information obtained from the foodstuffs and its consequences in clinical and medical problems is obvious. While this subject is out of the context of this chapter, it would be useful to remind that speciation of Co, Cr, Fe, Se and Zn in solutions for parental nutrition and oral supplements has recently become important from the point of view of bioavailability and toxicity.

11.2 Chromatography and Electrophoresis

Most of the speciation analyses are performed in samples where the analyte concentrations are very low and the matrices are complex. Therefore, the selectivity of many analytical techniques are forced to their limits under these conditions; interferences often play a degrading effect on the accuracy of results. Separation of analyte from the matrix can be performed in several ways such as distillation, precipitation, extraction, *etc*.; a brief information on these approaches was given in Chapter 4. The process of separation is often coupled to preconcentration that aims, in addition to the freedom from the matrix components, a higher final analyte concentration so that the chemical analysis can be performed at values significantly above the detection limit of the particular method. While the above-mentioned techniques of separation may often be successful in eliminating a heavy matrix, this approach may fail to separate the specific analyte forms from each other, proving to be inefficient when a speciation analysis is required. The ultimate separation technique should be able to separate

analytes from each other as well as from the complex matrix; this is commonly achieved by *chromatography* or *electrophoresis*.

Chromatography is the name given to a group of separation techniques that are also often coupled to a chemical analysis step. The process of separation involves the transfer and migration of analyte in a zone where the analyte is involved in equilibrium between two immiscible phases. One of these phases is usually coated as a thin film on an inert solid particle; this is called *stationary phase* (*SP*). The other phase is a liquid or gas and is called *mobile phase* (*MP*); this phase is moved continuously over the particles containing the SP. The particles with SP film are commonly packed into a *column* or are contained on a planar open surface such as paper or a thin layer smeared on a glass plate. These physical forms described above constitute a kind of classification and correspond to *column chromatography* and *planar chromatography*.

The analyte is introduced into MP in the chromatographic system. The stationary and mobile phases are selected in such a way that the analyte has an affinity to both phases in terms of an interaction such as solubility, adsorption and ion exchange, *etc*. As the analyte is distributed between the mobile and stationary phases, equilibrium is established as shown by the following expression:

$$K_d = A_{SP}/A_{MP} \tag{11.1}$$

where K_d is the *partition coefficient*, *partition ratio* or *distribution constant* and A_{SP} and A_{MP} are the analyte concentrations in the stationary and the mobile phases, respectively. While equilibrium is established for the analyte species between the SP and MP, the continuous flow of pure MP upsets this equilibrium since A_{MP} at any point will be reduced by dilution. This change forces A_{SP} to be also reduced in value since K_d is a constant for all the common cases of *linear chromatography* (see Section 11.2.1). As a result, the analyte species A is forced to migrate in the direction of MP flow. This equilibrium and migration is shown in Figure 11.1. The rate of this migration depends on the value of K_d; a large K_d means that the analyte A has a large affinity towards SP, therefore it moves at a lower rate as compared to other analyte species such as B and C with smaller respective partition coefficients. As a result, components of a mixture of species with very similar chemical structures will be separated using a chromatographic system along the direction by which MP moves.

When separation takes place on a plane chromatographic system, the spots corresponding to different analytes are visually inspected, wherever possible. If the spots are not visible, they may be rendered visible by using a reagent that will convert them into a visible or fluorescent form that may be viewed under daylight or an exciting source, such as a UV lamp.

On the other hand, the common way used in column chromatography is to place a detector at the end of the column. The separated species cause the corresponding signals as the analytes reach the detector; the resulting plot of signal vs. time is called a *chromatogram*, as shown in Figure 11.2.

The column chromatographic systems can be classified into several groups as shown in Table 11.1. The most general classification is made with respect to the physical form of MP. Therefore, the most general groups are named as *gas*

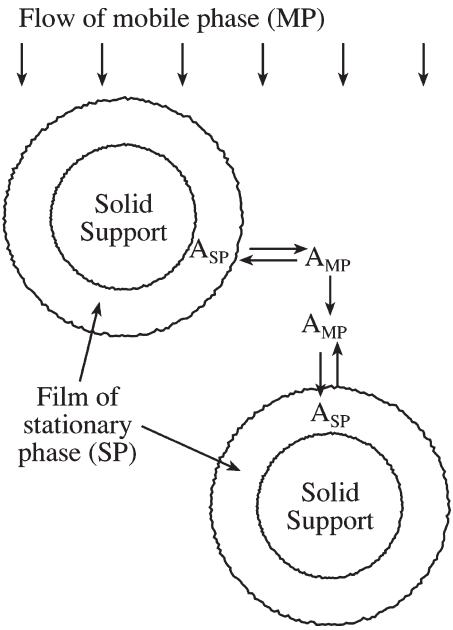


Figure 11.1 *Schematic illustration for the principle of GC. The example is given for the case where type of interaction is partition between gas and liquid*

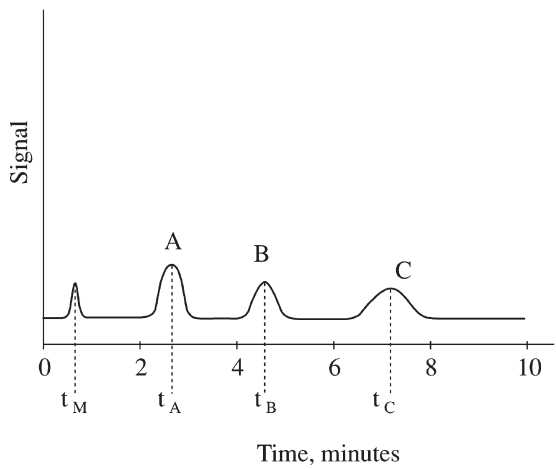


Figure 11.2 *A chromatogram showing the separation of species A, B and C with t_A, t_B and t_C as the corresponding retention times; t_M is the retention time for the mobile phase*

chromatography (*GC*), *liquid chromatography* (*LC*) and *supercritical-fluid chromatography* (*SFC*), with liquid, gas and supercritical fluid mobile phases, respectively. Capillary electrochromatography (CEC) with micelles as the pseudostationary phase is also a less common type of column separation technique.

Table 11.1 *Column chromatographic methods*[6]

General group	*Technique*	*SP*	*Type of interaction*
GC, MP: gas	Gas–liquid	Liquid adsorbed on a solid	Partition between gas and liquid
	Gas-bonded phase	Organic species bonded to a solid surface	Partition between gas and bonded surface
	Gas–solid	Solid	Adsorption and desorption on SP
LC, MP: liquid	Liquid–liquid, or partition	Liquid adsorbed on a solid	Partition between immmiscible liquids
	Liquid-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Liquid–solid, or adsorption	Solid	Adsorption
	Ion exchange	Ion-exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
SFC, MP: supercritical fluid		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface
CEC	MEKC or MECC	Micelles (pseudostationary phase) migrating in a buffer solution by electroosmotic flow	Partition between the buffer solution and the micelles
	Packed column electrochromatography	Low-polarity HPLC packing	Partition between the polar MP and low-polarity SP, electroosmotic pumping is used.

Electrophoresis is another method of separation followed by qualitative or quantitative detection; it is based on the differences in the migration rates of charged species in an electric field. Usually, 20–60 kV are applied between the ends of a plate or a capillary column; the analytes are introduced into an aqueous buffer solution. The species having larger charge to size ratio move faster while those with lower charge/size value move slower; the analytes reach the detector at different times and therefore the species in a mixture are separately detected. Classical electrophoresis has been performed on plates for rather qualitative purposes; this technique is called *slab electrophoresis*. Electrophoresis performed with capillary columns is a rather novel instrumental technique; this approach is called *capillary electrophoresis* (*CE*).

Another technique related to electrophoresis is the CEC. In this technique, the buffer solution adsorbed on the inner walls of the silica capillary is pumped by electroosmotic flow under a high electric field. CEC may be performed in a capillary column; the corresponding technique is called packed column electrochromatography. The more popular form of CEC is micellar electrokinetic capillary chromatography (MECC or MEKC). In this method, a surfactant is added to a buffer solution, and the surfactant concentration is high enough to form *micelles*. Micelles are spherical structures; in a polar solvent, the non-polar ends of the surfactant molecules are directed towards the centre of the sphere while the other polar ends are positioned on the sphere surface where the micelle is in contact with the polar solvent. Micelles are capable of dissolving non-polar species. Both the buffer and the micelles migrate towards one of the electrodes; buffer moves faster. The analyte species are distributed between the micelles (*pseudostationary phase*) and the separation takes place in a way similar to high pressure liquid chromatography (HPLC).

11.2.1 Common Laws and Properties for Chromatography and Electrophoresis

Both chromatography and electrophoresis produce signals as a function of the analyte concentration *vs*. time as the species pass through the detector; the plots of signal *vs*. time are called *chromatogram* and *electropherogram*, respectively. Signals are peak-shaped, and are often approximated to a Gaussian curve.

Some common rules and formulas have been derived in chromatography to illustrate, understand and control the performance of this technique. It must be noted that all these common formulas are based on the assumption that K_d is not dependent on analyte concentration in the system; in other words, the conditions for *linear chromatography* are assumed to exist. The derivation of these formulas will not be included here, but can be found in many textbooks.[6]

The performance of a chromatographic system depends on many variables such as the linear velocity of MP, diffusion coefficients for analyte in MP and SP, diameter of support particles, thickness of SP liquid coating on support particles and physicochemical characteristics of the analyte, MP and SP. However, the relations derived for chromatography are usually expressed in such a way that the information provided by a simple chromatogram can be used to reach the fundamental analytical figures of merit showing the performance.

Retention time, t_R, is the time elapsed for an analyte species between the introduction to the system and the arrival to the detector. A chromatographic signal is often characterized by its *retention time* (Figure 11.2) and the *peak width*, W_A (Figure 11.3).

The *length of column* packing is *L*. *N* is the *number of theoretical plates*. The *theoretical plate* is an imaginary section in the column at which the equilibrium is assumed to establish. Movement of analyte from one plate to the next is assumed as a stepwise transfer that is taking place in the discrete steps. *N* can be calculated directly from a chromatogram by using the following formula for all the eluted species and taking the average value of these results:

$$N = 16(t_R/W_A)^2 \tag{11.2}$$

Theoretical plate height, *H*, is the total number of plates in a column with a length of *L*:

$$H = L/N \tag{11.3}$$

Retention factor, k'_A, is measure of migration rate for the analyte A; it describes how well the rate for species A differs from that of MP. Ideally in a mixture of analytes, k' values should lie in the range between 2 and 10:

$$k'_A = (t_R - t_M)/t_M \tag{11.4}$$

Selectivity factor, α, is defined for two neighbouring species on a chromatogram and is a measure of the separation of these species:

$$\alpha = k'_B/k'_A = (t_B - t_M)/(t_A - t_M) \tag{11.5}$$

B is the species migrating more slowly than A through the column. Therefore, by definition, α is always greater than unity.

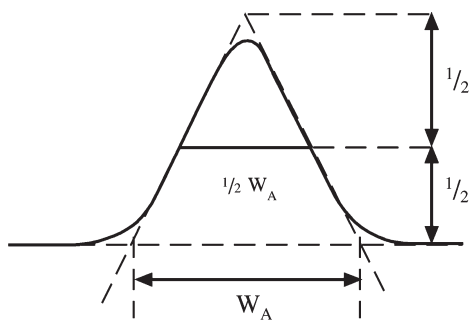


Figure 11.3 *Definition of a chromatographic peak.* W_A, *peak width;* $\frac{1}{2}$ W_A, *half peak width*

Peak broadening is a process in which W values get larger as the analytes are migrated through a column.

Resolution, R_S, is a measure of peak separation. High resolution is a desired property, so that the detector response to a species is well separated from the neighbouring ones. R_S value regarding the two sequential signals, A and B, can be defined as follows in terms of retention times and the peak widths:

$$R_S = 2(t_B - t_A)/(W_A - W_B) \tag{11.6}$$

As can be seen, the retention times with well-separated values will result in a better resolution while a larger peak width will degrade it.

Mobile phase flow rate, u, can often be measured directly by monitoring t_M on a chromatogram or by using a flow meter connected to the output of a column, as usually done in a gas chromatograph. It must be experimentally optimized to give the best column resolution.

The purpose of a chromatographic separation is to introduce the separated analytes to a detector in such a way that their signals should be well differentiated from each other and yet the total consumed time for this process should be a minimum. Therefore, all the optimizations performed on a chromatographic system aims at the best resolution in a minimum elapsed time. In this optimization, some opposing factors must be considered. For example, a longer column will contain higher number of theoretical plates, N, but on the other hand the peaks will get broader as they are eluted for a longer time through a column. In connection with these factors, the mobile phase flow rate must have an optimum value.

Both qualitative and quantitative analyses can be performed by utilizing a chromatogram. Under well-defined chromatographic conditions, retention time values are specific to each analyte; thus a qualitative analysis is practically possible solely by observing the retention times on a chromatogram. On the other hand, the peak height or peak area can be used for quantitative analysis by using standard signals and a calibration plot.

Although an electropherogram and a chromatogram look very similar, the principles of separation differ in many respects for these techniques. The difference in the rate of migration under an electric field has been known as a technique of separation for many years; the *slab electrophoresis* is still applied on plates in many laboratories for qualitative purposes. Using the principles of electrophoresis in a capillary column, however, is a rather novel technique that has been developed in the last two decades. Although this type of separation involves a column, the technique cannot be termed as chromatography since there is no SP in this system. Separation is realized in an applied electric field and is based on the different migration velocities for the analyte species attracted to one of the electrodes, the differences depend on the charge to size ratio of the species.

The velocity of a species in an electrophoretic process is proportional to the electric field strength E (in V cm^{-1}) and the electrophoretic mobility μ_e (in $cm^2\,V^{-1}\,s^{-1}$), where V is the applied potential (in v) and L the length (in cm) over which the voltage is applied:

$$v = \mu_e E = \mu_e(V/L) \tag{11.7}$$

Electrophoretic mobility is proportional to the charge on the species. In addition, during the migration of species in the capillary column, there will be frictional forces that will reduce the velocity; these forces are proportional to the size of the species. Therefore, the net resulting velocity, considering both Equation (11.7) and the frictional forces, will be proportional to charge to size ratio. According to Equation (11.7), high-applied voltages will result in shorter periods of analysis. A high resolution is also required for an electropherogram. Band broadening in chromatography is caused by both the longitudinal diffusion and mass transfers in MP and SP. Since there is no SP in electrophoresis, mass transfer is not a factor and thus only the longitudinal diffusion causes the band broadening or degradation of resolution. Therefore band broadening in electrophoresis is less than that in chromatography. The number of theoretical plates in electrophoresis is given by the following expression:

$$N = \mu_e V/2D \tag{11.8}$$

where D is the diffusion coefficient of solute in $cm^2\ s^{-1}$. An important property of electrophoresis is that N is not proportional to L: using longer columns for better R_s is not necessary.

For slab electrophoresis and CE, typical applied voltages are 500 V and 20–60 kV, respectively. CE has plate counts in the range of 100 000–200 000. According to Equation (11.7), the analyte species introduced into a capillary filled with a buffer solution will acquire different velocities under the applied electric field; the migration induced under these conditions is called *electrophoretic flow*. Another kind of migration is induced when the inside surface of the silica capillary has a positively charged ionic layer, which is pulled to the cathode end, bringing the other solvent molecules together; this type of migration is called *electroosmotic flow*. Silica surface contains acidic Si–OH groups which dissociate around pH 3 and thus become negative; positive ions in the buffer are adsorbed on this surface, forming a dense charged layer, which is the cause of electrical attraction in electroosmotic flow. While the electrophoretic flow is the basis of electrophoresis, electroosmotic flow is used in electrochromatography. In some cases, both of these flows may be effective; in other words, electroosmotic mobility, μ_{eo}, is also a determining factor on the velocity:

$$v = (\mu_e + \mu_{eo})E \tag{11.9}$$

Regarding the velocities towards a negative electrode, μ_e for anions will have a negative sign. In such a case, the anions will move towards the negative electrode only if the electroosmotic flow is stronger than the electrophoretic flow.

Adding a cationic surfactant that will be adsorbed on the silica wall and thus rendering the surface charge positive may reverse the direction of the electroosmotic flow. In this case the ions forming the intensely charged layer near the wall will have negative signs and the electroosmotic movement will take place towards the anode. When it is necessary to eliminate the electroosmotic flow, using a reagent such as trimethylchlorosilane that will form a coating on the surface may deactivate the silica surface. The surface having this type of coating will not have its charged surface

groups exposed, the intensely charged layer on the wall and thus the electroosmotic flow will cease to exist.

11.2.2 Instruments for Chromatography and Electrophoresis

An instrument for chromatography or electrophoresis consists of two important components; serving for separation and detection of analytes. A column or a plane containing the SP serves as the component for separation. At the end of the separation zone, there is a detector. Other devices such as pumps, valves, *etc*. help to drive the MP through the separation zone, introducing the sample, varying solvent composition or temperature. Since most of the modern separations are carried out in columns, we will concentrate on these systems.

11.2.2.1 Columns

A chromatography column is defined by its internal diameter, length, SP and the way this SP is contained in the column. If the SP is coated as a thin film on inert solid support particles that are filling a metal or glass column, this system is called a *packed column*.

LC employs packed columns. The column efficiency is better with smaller particle size; however this causes a backpressure or a resistance to MP flow. This resistance is overcome by applying a pressure on MP, ranging in between 500 and 10 000 psi. This applied pressure is the reason for the name given to these systems, HPLC. LC columns are made from smooth-bore stainless-steel tubing or heavy-walled glass tubing. For applications requiring pressures higher than 600 psi, steel tubing must be used. The length of HPLC columns range in between 5 and 30 cm; internal diameters are often 4–10 mm; the most common particle diameter is 5 or 10 μm. Recently, packing materials with particle sizes as low as 3 μm have been produced.

There are a large variety of column materials for different problems of separation. In the earlier applications of HPLC, a highly polar SP and an MP of medium polarity were used; this was named as *normal phase chromatography*. Later on, the use of a low-polarity SP with an MP of medium polarity, usually aqueous, became more common; this configuration has been named as *reverse phase chromatography*.

In some cases, MP has a defined composition that does not change throughout the chromatogram, and such a system is termed *isocratic*. Alternatively, MP may consist of two miscible solvents whose ratios are altered throughout the chromatogram to facilitate a more efficient separation; this can be done by using two pumps for the corresponding solvents. Flow rates and therefore the ratio of solvents in MP are altered in such a way that the total flow rate is constant. This approach is called *solvent programming*. Solvent programming is designed accordingly to obtain the best chromatogram in shortest time. In order to do the same in GC, temperature is varied while a chromatogram is obtained and is called *temperature programming*. Therefore, *solvent programming* and *temperature programming*, although physically totally different processes, are the approaches used for the same purpose, an efficient separation in shortest period, for HPLC and GC, respectively.

The degree of the interaction of analyte with MP and SP is variable and may approach to zero in some occasions. For example, the MP or carrier gas in GC acts

only as a transporter of analytes; use of different carrier gases, such as helium, nitrogen or hydrogen, does not significantly affect the separation process. On the other hand, the analytes are dissolved in MP for the HPLC systems; altering the composition of MP significantly affects the efficiency of separation.

GC uses both *packed columns* and *capillary columns*. In the latter, SP may be coated on the interior surface of the column tubing; such a configuration may also be called as *open tubular column*. Open columns have much better resolutions as compared to the packed columns and have gained a general acceptance in laboratories. Open tubular columns are also classified regarding the manner the SP coating is applied. The following are the most common types:

- WCOT (wall-coated open tubular columns)
- SCOT (support-coated open tubular columns)
- FSOT (fused-silica open tubular columns)

The length of GC columns vary between 1 and 100 m and the internal diameters are in the range of 0.1–4.0 mm. Mobile phase or carrier gas must be chemically inert; He, N_2 or H_2 are commonly used.

In a chromatographic process matrix components of the analytical mixture may have degrading effects on the column. Particulate materials may clog the column and some components may be irreversibly adsorbed on the SP, thereby modifying its behaviour. In HPLC, a short column is placed before the analytical column to protect it from the negative effects. This short column is called as *guard column*. The composition of the guard column packing is similar to that of the analytical column. As the MP passes through the guard column, the solvent is saturated with the SP, so that the SP film in the analytical column is not eroded in a short period; this is another function of the guard column to protect the analytical column. Naturally, the guard columns are renewed often as they are contaminated so that the lifetime of the analytical column is improved.

Both open tubular and packed columns are used in SFC; column lengths are 10–20 m. Open tubular columns have typically an internal diameter of 0.05 or 0.10 mm; the packed SFC column internal diameters range in between 0.5 and 4.6 mm. Particle size of packing material is in the range of 3–10 μm. The stationary phases are similar to those used in HPLC.

11.2.2.2 Detectors

In GC, there are a number of commercially available detectors. A gas chromatograph can be purchased with several of these commercial detectors.

Flame ionization detector (*FID*) is a widely used sensitive GC detector. Analyte is ionized in a hydrogen-air flame; and the produced ions result in a current that is measured and correlated to the number of analyte molecules. FID, therefore, is a *mass-sensitive* detector rather than a *concentration-sensitive* transducer. Most organic species can be detected. FID has a general (universal) response to organic molecules.

Thermal conductivity detector (*TCD*) is based on the principle of gas conductivity measurements performed in a flow. The carrier gas or pure MP thermal conductivity is

continuously measured in a reference channel while the stream containing analytes is subjected to a similar measurement in a sample channel; the difference corresponds to the changes caused by the analyte species. The common carrier gases such as He and H_2 have relatively high thermal conductivities; the presence of organic analytes significantly lowers the thermal conductivity, causing the formation of analytical signal. TCD is less sensitive than FID; however, it is non-destructive while FID is destructive.

Sulfur chemiluminescence detector (*SCD*) is specific to sulfur-containing analytes that are burned in a hydrogen-air flame; products of combustion are mixed with ozone to form chemiluminescent species. The resulting light emission is correlated to analyte concentration.

Electron capture detector (*ECD*) has a wide range of use especially for environmental samples containing halogens, such as some pesticides and polychlorinated biphenyls (PCBs). This detector contains a β emitter, usually nickel-63. Electrons emitted ionize the carrier gas, and a steady current is thus established. Organic analyte molecules may capture these electrons; and the decrease in the current is the analytical signal. Halogens, peroxides, quinones and nitro groups are some common analytes for ECD.

Atomic emission detector (*AED*) consists of a microwave energized He plasma in which the intensity of atomic emission from the analyte elements are measured using a diode array polychromator.

Thermionic detector (*TID*) is selective to organic compounds containing P and N. The analytical signal is caused by large ion currents resulting from an electrically heated rubidium silicate bead, as P or N containing analytes pass through the detection zone.

Infrared (*IR*) *spectrometry* and *mass spectrometry* (*MS*) as detectors have been successfully coupled to GC systems, resulting in powerful hyphenated techniques such as GC–IR and GC–MS.

In addition to commercially available detectors, combinations between GC and atomic detectors, such as AAS, AES, inductively coupled plasma (ICP)-AES and ICP-MS, have been the popular subjects in many research laboratories. These unconventional detectors will be discussed later in this chapter.

In HPLC, the output from the analytical column is a solution; therefore, in principle any analytical technique used for liquids can be successfully employed as a detector. An important consideration is the detector cell design where the analyte dilution and peak broadening should be minimized. A *Z-shaped detector* cell design is shown in Figure 11.4; this is a configuration that provides minimum band broadening in the detector cell.

Optical detectors based on the principles of UV, VIS or IR absorbance, fluorescence, refractive index and light scattering are commercially available. Other commercial detectors are electrochemical, conductivity and mass spectrometric systems. In a way similar to GC, coupling of HPLC to common atomic spectrometric systems such as AAS, ICP-AES and ICP-MS is a popular research subject that will be handled later in this chapter.

Almost all the HPLC detectors can be used in SFC; in addition, FID can also be used. Availability of FID as a very sensitive and general response detector is an advantage of SFC over HPLC.

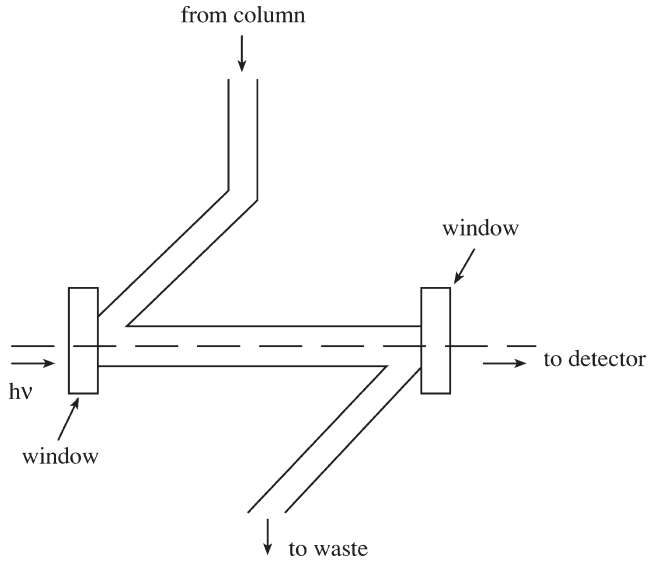


Figure 11.4 *A typical Z-shaped flow detector cell for UV or VIS absorbance measurements for HPLC*

CE detectors should handle very small volumes, of the order of nL. The detector is placed near the electrode to which the migration of species takes place. The detector cell is designed to enlarge the volume of the capillary section in the measurement zone. For absorption measurements, a *bent capillary* is forced to lie along the light beam or a *bubble* in capillary tube is made, or the source light beam is subjected to *multireflections* on mirror coated internal capillary surface to have a longer path length of radiation. Laser excitations in fluorescence measurements have yielded very high mass sensitivities. Electrochemical detectors have also been used. For mass spectrometric detection, a special form of sample introduction is used; the liquid emerging from one end of the capillary is forced to form a charged spray in ambient temperature and pressure; the solvent is evaporated and the charged particles are directed into the mass spectrometer. This technique is called *electrospray ionization*. The end of the capillary is metalized by a coating, a ring metal electrode is placed around this tip and a potential of several kV is applied between these two electrodes to effect the electrospray action. This method is successfully employed to sample large biomolecules, such as proteins, polypeptides and oligonucleotides having molecular weights of $\geq$100 000 Da. The importance of this technique for a large variety of food and diet samples is obvious.

The small volume capacity of CE detectors is a problem in obtaining the proper concentration sensitivity. For example, a mass detection at the level of pg is impressive; however, if the volume is of the order of nL, the concentration will correspond to pg nL^{-1} or mg L^{-1}, which is relatively high for many analytical problems. Laser-excited fluorescence has been used[7] to reach a mass detection as low as 6 zm (10^{-21} mol) for cysteine that was labelled with fluorescein isothiocyanate; this corresponds to a concentration level of 5.6×10^{-12} M.

11.3 Typical Instruments

Some of the typical instrumental designs are shown in Figures 11.5–11.8 using schematic representations.

Typical chromatograms[8] of a rice flour SRM obtained by different sample preparation techniques and ion chromatography inductively coupled plasma-mass spectrometry (IC-ICP-MS) are shown in Figure 11.9.

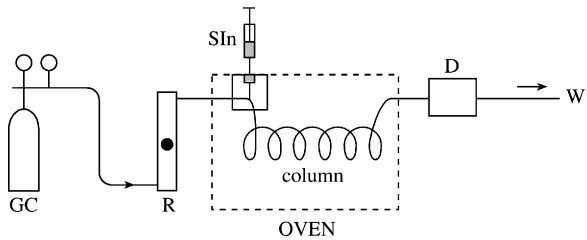


Figure 11.5 *Gas chromatograph. GC, gas cylinder; R, rotameter to regulate gas flow; SIn, sample injection port; D, detector; W, waste*

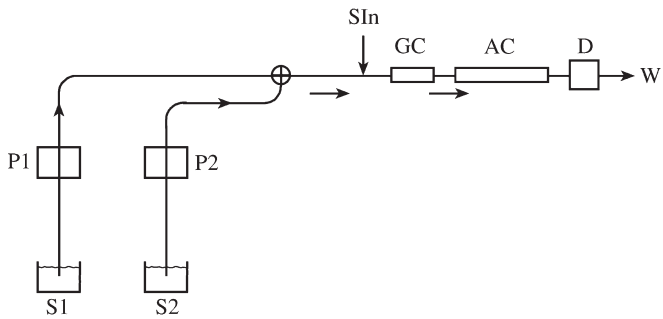


Figure 11.6 *High-pressure liquid chromatograph. S1, S2, solvents; P1, P2, pumps; SIn: sample injection; GC: guard column; AC: analytical column; D: detector; W: waste*

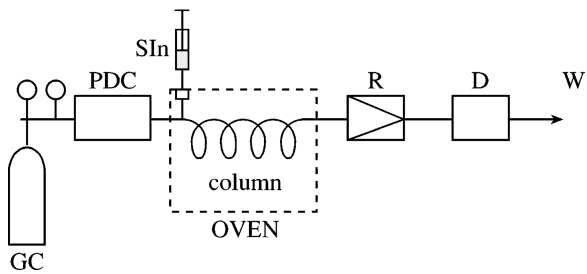


Figure 11.7 *Supercritical-fluid chromatograph. CG, carrier gas; PDC, pressure and density control; SIn: sample injection; R: restrictor; D: detector; W: waste*

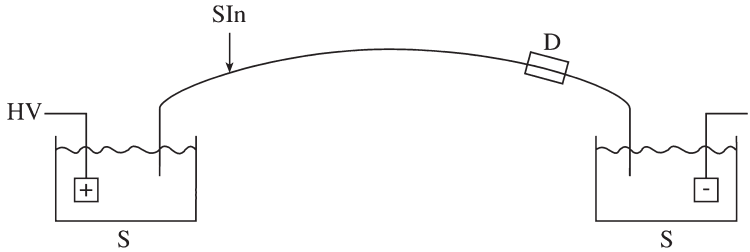


Figure 11.8 *Capillary electrophoresis instrument. HV: high voltage; S: solvent; SIn: sample injection; D: detector; S:solvent*

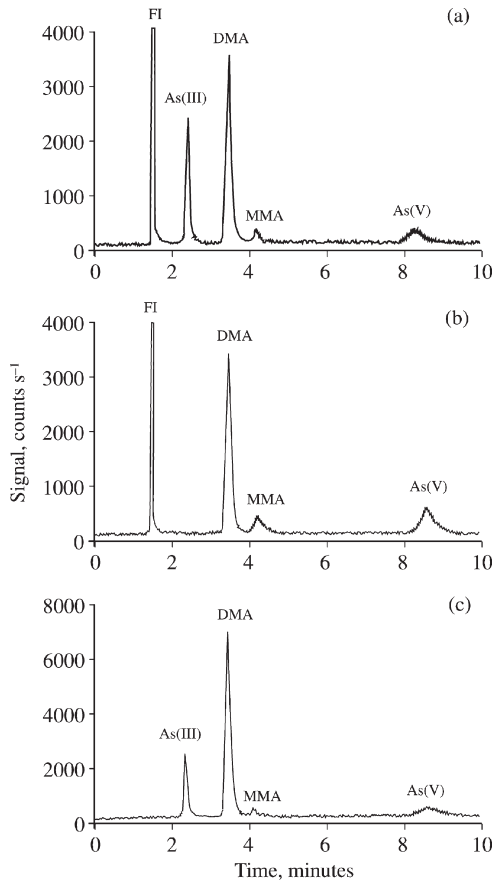


Figure 11.9 *Chromatograms of SRM 1568a rice flour using ion chromatography-inductively coupled plasma-mass spectrometry (IC-ICP-MS). (a) TFA extraction; (b) amylase/50% methanol extraction; (c) 50% methanol with sonication. FI, 5 ng As mL^{-1} flow injection peak; DMA, dimetylarsinic acid; MMA, methylarsonic acid*

References

1. J. Versieck, L. Vanballenberghe, A. Wittoek, G. Vermeir and C. Vandecasteele, *Biol. Trace Elem. Res.*, 1990, **26–27**, 683.
2. G.F. Harison, in *Lead in Marine Environment*, M. Branica and Z. Konrad (eds.), Pergamon Press, Oxford, 1980.
3. C. Alzieu, J. Sanjuan, P. Michel, M. Borel and J.P. Drene, *Mar. Pollut. Bull.*, 1989, **20**, 22.
4. D.M. Templeton, F. Ariese, R. Cornelis, L.-G. Danielsson, H. Muntau, H.P. Van Leeuwen and R. Lobinski, *Pure Appl. Chem.*, 2000, **72**, 1453.
5. D. Behne, *Analyst*, 1992, **117**, 555.
6. D.A. Skoog, F.J. Holler and T.A. Nieman, Principles of Instrumental Analysis, 5th edn, Saunders College Publishing, Philadelphia, PA, 1998.
7. S. Wu and N.J. Dovichi, *J. Chromatogr. A*, 1989, **480**, 141.
8. D.T. Heitkemper, N.P. Vela, K.R. Stewart and C.S. Westphal, *J. Anal. Atom. Spectrom.*, 2001, **16**, 299.

Further Reading

1. A.M. Ure, C.M. Davidson (eds.), *Chemical speciation in the environment*, Blackie Academic and Professional, London, 1995.
2. P. Quevauviller, *Method Performance Studies for Speciation Analysis*, The Royal Society of Chemistry, Cambridge, 1998.

Chapter 12

Comparison of Analytical Techniques

12.1 General Approaches for Selecting a Technique

There are two main approaches for selecting an analytical technique. The first one is to determine the analytical tasks and targets, then to purchase the most suitable instruments that will allow the users to obtain the desired results easily and with sufficient quality. This is the ideal way and thus must be followed whenever the budgetary conditions allow. The second approach is to see what instruments are available or what equipment could be purchased; then the analytical capability of laboratory, tasks and targets is accordingly determined. Although the first approach is ideal as stated above, unfortunately the laboratories in many countries may have to follow the second path because of limited budgets. In this case, if the desired analytical quality is not readily achieved using the instruments available, further effort such as preconcentration, matrix-separation, *etc*. may be necessary. For instance, if only AAS instruments are available, the users will have to develop special techniques to obtain the sensitivity that could be readily provided by a rather more costly ICP-MS system. Budgetary deficiencies may, therefore, be compensated in some cases by spending more effort in laboratory.

In some cases, availability of technical facilities may be decisive. The presence of a nuclear reactor nearby and the availability of using it in an economical way may direct the laboratory personnel to purchase instrumentation for INAA, a very capable technique for most elements.

In all cases, a balance will have to be established considering the financial sources and analytical tasks required. Nevertheless, the basic knowledge for comparison of analytical techniques would be very useful to give correct decisions in order to purchase the proper chemical-analysis instruments in trace element analysis of food and diet samples. In this chapter, some criteria for selecting analytical techniques will be discussed along with the properties of techniques in several fields for this comparison.

12.2 Criteria for Selecting an Analytical Technique

Before a detailed comparison of analytical techniques, it is useful to discuss about some common criteria. The numbers related to these performance criteria are sometimes

called *analytical figures of merit*, as discussed in Section 3.2.5. Prior to purchasing a new instrument, some estimations can be made; true figures require an extended use and experience on a specific analytical system. It is always useful to accept suggestions from several sources of competence.

12.2.1 Considerations for Sample Preparation

The techniques of XRF and INAA require solid samples in general. Solutions are best for flame and electrothermal AAS, ICP-OES and ICP-MS; in addition to nebulization, the other common sample introduction techniques in this group, namely hydride generation and cold vapour formation, also require liquid samples. Although there are exceptions and continuous research to use the less preferred physical state on both sides, the practical and most general trends are as stated above. Therefore, laboratories using AAS, ICP-OES and ICP-MS should be well equipped with the tools for effective sample handling, such as microwave ovens and other systems, for dissolution and decomposition. If the sample must be dissolved, the time and effort to be spent for sample preparation would be naturally higher as compared to solid sampling for corresponding techniques.

On the other hand, methods requiring solid samples are also associated with some sample preparation. For INAA, powdered samples must be placed in quartz or hard plastic tubes that should be sealed afterwards. Solid samples are often fused and pressed as a pellet prior to analysis by using XRF.

In some cases, only a limited amount of the sample is available. A very small quantity of tissue or body fluid in a medical problem, a tiny sample for archaeological object or a small sample for evidence in a forensic issue is a typical example. In food and diet analyses such cases are very rare; there is practically no limit for sample quantity in general. Therefore, any advantage regarding the use of very small sample sizes, such as the case of ETAAS, is not relevant in food analysis; obviously, ETAAS may be the technique of choice for other reasons, such as sensitivity.

Homogeneity problems are to be considered during sample preparation in certain occasions. Determination of Au in quartz minerals is a typical case. Since this element is present as pure metal particles rather than being evenly distributed as a chemical compound, at least 10 g of sample must be dissolved to obtain a good sampling average and thus a representative sample. In such occasions, it does not matter how sensitive the technique is, the sample size cannot be reduced. The employment of direct solid samples using XRF or INAA would not be recommended for such a problem. Regarding food and diet analysis, most of the samples are homogeneous. However, the sampling problem above should be kept in mind.

The final matrix of aqueous samples should be compatible with the technique to be used. In most classical dissolution procedures, the solution is evaporated near dryness, then the contents are re-dissolved using minimum amount of reagent that would result in a solution with a simplest matrix. When closed PTFE vessels are used, this approach is rarely applied. Therefore, maximum effort should be spent to obtain a matrix that would cause minimum interference problems during final determination. In general, HNO_3 is a preferred acid for AAS, ICP-OES and ICP-MS.

12.2.2 Sensitivity

Although the concept of sensitivity may be referred to and evaluated by several definitions such as calibration and analytical sensitivity, signal to noise ratio, limit of detection (LOD) and limit of quantitation (LOQ), the most widely used one is LOD. The lower end of working range is practically defined by LOQ that is about 5–10-fold of LOD. In most cases, LOD of instrument in your laboratory may be about 10 times higher than that was given by the manufacturer. There are many reasons for this situation among which are the age and condition of equipment, intensity of source lamps in case of AAS, efficiency of nebulizers, *etc*. The manufacturer's LOD values may be obtained with very simple matrices and/or using time periods longer than what is normally used for a routine measurement. Typical limits of detection to be expected in several analytical techniques are given in Figure 12.1.

Higher detection limits are obtained in complex matrices. It is seldom that an analyst has to determine trace elements in almost pure water that has a very simple matrix. Increasing matrix complexity causes deterioration of precision in all steps

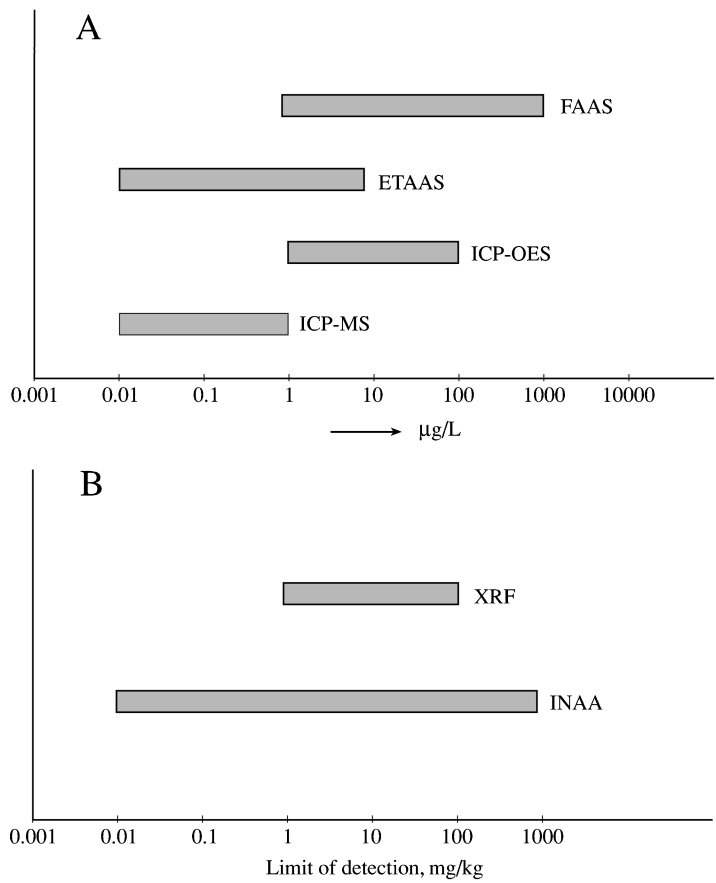


Figure 12.1 *Limit of detection values for analytical techniques, A: µg L^{-1}, B: µg kg^{-1}*

such as dissolution, sample transport, nebulization and final detection. It should be remembered that reduced precision causes lower sensitivity and higher LOQ values. A good rule of thumb would be to estimate an LOQ value of 100-fold of that given by manufacturer; application of the full method for a particular analytical problem will yield true LOQ. Nevertheless, in many cases, analyte concentration is well above LOQ values; in this case sensitivity is not the primary criterion.

12.2.3 Speed

Regarding speed of an analytical technique, most often the term *sample throughput* is used and the number of samples analysed per unit time is thus indicated. Although, sometimes this term may be defined for number of samples measured, the more realistic estimation for the sample throughput must be made by considering the total time required for sample treatment, analysis and data evaluation. It is a well-known fact that in most cases the time required for sample preparation is longer than that needed for determination and data evaluation. The period of time necessary for data evaluation, thanks to computing software facilities, has become almost negligible as compared to total analysis time. For example, an atomic spectrometric technique would require at most few minutes to determine and report analyte concentration; however, sample treatment may need hours in some cases.

For techniques where nebulization is used for introduction of solution samples, *wash-in* and *wash-out* periods are important, before and after data acquisition, respectively. Therefore, these two periods are included in total sample measurement period. A complete wash-out is necessary so that the measurement zone will be cleansed off the last sample and thus no *memory effect* will take place. At this point, sample uptake rates for nebulization become important. For FAAS, sample uptake rate is around 5–6 mL min^{-1}. Rinsing with solvent, mostly pure water, is not commonly required as the wash-in and wash-out times are short as compared to data acquisition period. Consequently, time required for a single measurement is relatively short, 5–30 s depending on *S*/*N*. On the other hand, during nebulization into Ar plasma for ICP-OES or ICP-MS, sample uptake rate is around 1.0 mL min^{-1}, significantly slower as compared to FAAS. A single measurement thus may take as long as few minutes including rinsing with solvent between the samples; and this is usually required. Although this comparison is valid for a single measurement for one element, the multi-element capability of ICP-OES and ICP-MS are far superior as compared to AAS. Overall speed for ICP-OES and ICP-MS is then higher than that of AAS when multi-element capability of Ar plasma techniques are used.

A simultaneous ICP-OES, XRF, INAA and multi-element AAS (only up to 6 elements as of today) and ICP-MS have the advantage of multi-element capability over single-element AAS or sequential ICP-OES instruments. A quadruple ICP-MS is actually a very fast scanning instrument while TOF-ICP-MS can be considered as totally simultaneous with some advantage over the former regarding multi-element capability. Small differences between the fast scanning systems become more important for discrete sample introduction.

A wide dynamic range is always preferred. In the case of a short dynamic range, further dilution will be required for samples with analyte concentrations exceeding

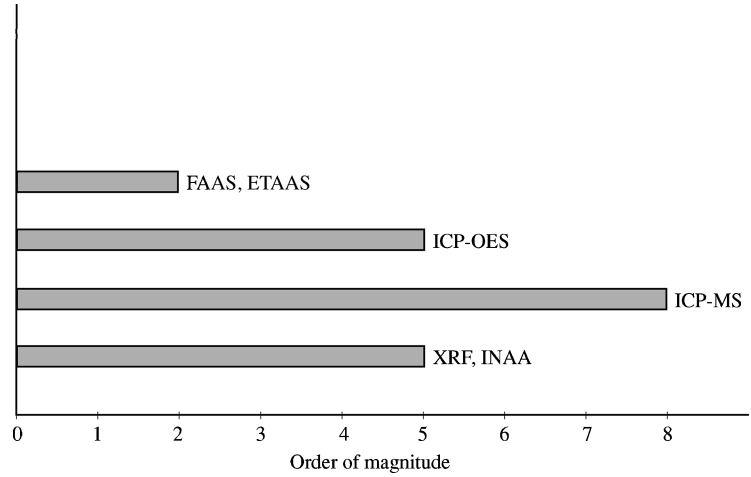


Figure 12.2 *Dynamic ranges, order of magnitude*

the upper limit. This is often experienced in AAS analyses where the dynamic range is only one or two orders of magnitude. A wide dynamic range reduces this undesirable possibility or eliminates it totally in some cases. Therefore, wide dynamic ranges, even more important in multi-element systems, result in shorter analysis times. Typical dynamic ranges for analytical techniques are given in Figure 12.2; the superior value associated with ICP-MS should be noted.

12.2.4 Ease of Use

It is often suggested that "the lack of need for an expert" is an advantage for an analytical technique. Lower salaries for non-experts will reduce personnel costs. Although such a feature may seem as a valid way to lower the analysis costs, we believe that the presence of laboratory personnel with experience and knowledge will always pay in the long run. It must be remembered that a human being with a good judgement is the most valuable element in a laboratory.

On the other hand, it may be suggested that if much attention is not required during analysis, the personnel may use the time made available for other activities such as sample treatment, data reduction, report preparation, reading, *etc*.

Ease of use may be a relative concept at times. If a laboratory has the availability of a nuclear reactor in close neighbourhood, using INAA may become very convenient and advantageous. Similarly, laboratory personnel who are knowledgeable about XRF will always prefer another XRF system when there is need for new instrumentation.

Each analytical technique has different aspects regarding ease of use. We already discussed the sample preparation above. In most cases, this step is not only the most time consuming, but also the most difficult stage of analysis. Therefore, sample preparation procedures required should always be considered regarding ease of use for any analytical technique.

Once the difficulties with sample preparation are overcome, there remain basically three main activities regarding the ease of use:

- Using instrument for data acquisition
- Calibration, maintenance, changing parts and accessories
- Interpretation of results, data evaluation, error analysis, detecting the presence of interferences

The first one, using instrument for data acquisition, is probably the most critical task; however, the proper handling of the other two tasks is also absolutely needed for analytical results of high quality.

Some techniques, such as XRF and INAA, can be used without any special effort during data acquisition. Others, atomic spectrometry and ICP-MS, are also frequently equipped with powerful sampling automation systems. However, since the sample transport to atom cell is a critical step, user attention is often required. Some measurements may be performed by manual sample introduction in AAS and ICP-OES, especially during initial checks, optimization of parameters and method development. Automation not only provides speed, convenience and comfort but also improves precision. A typical and well-known case is sample injection into cuvette for ETAAS; manual sampling is possible and may be used at times, but automated injection results in definitely better precision. Using flame AAS, ICP-OES and ICP-MS, introducing a liquid sample is accomplished by very similar techniques of nebulization.

Vapour generation techniques, on the other hand, such as HGAAS and CVAAS, form another group for handling liquid samples for AAS, ICP-OES and ICP-MS, where sample transport may be more intriguing as compared to nebulization. Although vapour generation techniques are almost as old as using a flame atom cell, generation vessels, gas–liquid separators and other tools for producing analyte vapour have not been standardized. Consequently, there are no standard apparatuses for vapour generation techniques where flame atomization or Ar plasma systems are very similar in different instruments. Therefore, these methods require user attendance and care particularly in method development stages.

In addition to the need for a careful optimization of conditions by well-attended experiments, even automated systems require some attention on several aspects such as the condition of tubing for flow sampling systems that is vital for a reproducible sample transport into reaction zone and therefore analyte transport into the signal measurement zone.

Calibration, maintenance, changing parts and accessories can be made quite easily in most analytical techniques discussed here. Although some of these tasks are the duties of service personnel, an able analyst in the lab is usually able to handle these problems.

Regarding the last group of actions, interpretation of results, data evaluation and error analysis, the potential presence of interferences plays an important role. From this point of view, flame AAS and AES techniques have a rather highly diluted sample in atom cell due to low nebulization efficiency. Potential interferants are also diluted and thus minimum level of interferences is encountered in these techniques. Although the use of flame for AES is limited to only few alkali and earth alkaline elements, such as Li, Na, K, Ca, Rb and Sr, this atom cell is cooler as compared to

Ar plasma. The result is lower level of spectral interferences in flame because many species are not sufficiently excited in the relatively cooler atom cell.

ETAAS, on the other hand, has an atomizer where analyte and potential interferants in matrix are not subjected to much dilution. Therefore, the increased sensitivity for analyte is always accompanied by chemical and spectral interferences; the latter is observed mostly in the form of background absorption. Negative effects of these conditions on precision and accuracy should be minimized by a careful selection of temperature programme and use of chemical modifiers. As a result, skilful and experienced operators are required for ETAAS. Experience, knowledge and intuition are required especially for analysis of *new* samples with complex matrices. Fortunately, articles with good, guiding suggestions regarding ETAAS problems are now plenty in literature. In some occasions, however, there may be conflicting reports. Differences may be caused by different instrument and cuvette designs. The user should therefore make a careful choice while evaluating advice from literature.

Laboratory safety is another important issue of common concern. Safer systems are easier to handle. Possibility of having accidents is lower. INAA and XRF users are subjected to some radiation, necessary precautions should be taken. Flame AAS laboratory personnel must use flammable gases and are subjected to more severe safety problems as compared to those employing techniques such as ETAAS, ICP-OES and ICP-MS where only inert gases are used.

12.2.5 Cost of Instrumentation and Analysis

Cost of chemical analysis is normally well justified in a system working efficiently, producing analytical results of high accuracy and desired precision. If data of high quality are presented timely to the parties to use these results, relative cost is minimized. On the other hand, results of low dependability, late-produced results and use of laboratory inefficiently are elements and events that increase the cost of running.

One initial concern is the price of a new analytical system. Resources are often supplied for well-justified projects. Nevertheless, financial resources may be scarce at initial steps for purchasing an instrument.

Purchasing an INAA system is justified if a well-running nuclear reactor is available with a reasonable cost of use. Prices of systems for counting radiation range in 100 to 500 K€ . XRF systems may be preferred for analysis of solid samples, since sample preparation step will not include dissolution. High performance XRF systems may cost as high as 500 K€ . Running costs are maintenance and X-ray radiation tubes having a life-time of several years.

Flame photometers can be used for determination of only few elements such as Na, K, Li, etc. Their price is as low as 5000 € and the cost of flame gases is rather low; most laboratories today use natural gas, that is almost pure CH_4 and air. If this instrument of rather limited use is excluded, flame AAS has the lowest cost among the atomic spectrometric techniques. A flame AAS instrument of reasonable quality costs around 40 000 € . Need for additional ETAAS capability approximately doubles the cost. The apparatuses for vapour generation systems cost in a range of 5000–20 000 €; however, these systems can be manufactured in lab at much lower costs using a peristaltic pump and glass shop facilities.

Among the running costs for AAS are hollow cathode lamps (200–800 €); their lifetimes may be as low as few months; whereas some of them may be used for years. Their expected life-times are usually given by manufacturers in units of ampere-hour. Flame gases such as acetylene are usually locally available at rather low costs, a good air pump is necessary. For ETAAS, a graphite cuvette costs around 50 €. Depending on the skill of the user and properties of sample matrix, a cuvette's life ranges between few uses and about 1000 firings. While the lifetime of a cuvette is usually well known for established routine uses, during the method development stages they may be destroyed at early stages.

A sequential ICP-OES instrument costs around 100 000 €. A simultaneous ICP-OES instrument may be purchased for 150 000–200 000 €. Because of the recent developments in array detector technology and consequent ease of multi-wavelength measurements, it is expected that all ICP-OES instruments in near future will be of the type simultaneous; their prices may be lowered.

Basic running cost of ICP-OES is due to the consumption of Ar gas that is needed at a flow rate of 10–20 L min^{-1}. The cost of Ar gas may be easily afforded for a laboratory producing high amounts of analytical results in a day. However, it is well known that this cost may be prohibitive in some developing countries. This is one of the important reasons why single-element AAS still survives as an analytical technique despite the superior performances of ICP-OES and ICP-MS.

ICP-MS instruments have a price range of 150 000–500 000 €. Elements of running costs are, in addition to Ar gas, sampling and skimmer cones placed at the entrance of mass spectrometer section of this instrument. Since both ICP-OES and ICP-MS use the same atom cell, Ar plasma, running costs regarding Ar are basically the same for these systems. Plasma torches also need to be replaced in few months, the cost is 350–1500 €.

A summary of instrument costs for several analytical techniques are given in Figure 12.3.

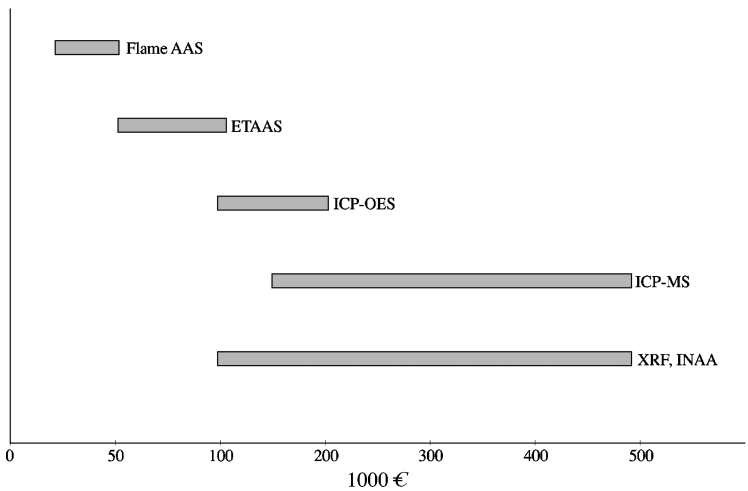


Figure 12.3 *Cost of instruments, 1000 €*

Table 12.1 *Comparison of analytical techniques*[a] *based on practical analysis criteria*

Criterion	*FAAS*	*ETAAS*	*ICP-OES*	*ICP-MS*	*XRF*	*INAA*
Typical LOD ($\mu g\ L^{-1}$)	1–1000	0.1–10	1–100	0.01–1	1–100 mg kg^{-1}	0.005–1000 mg kg^{-1}
Dynamic range, orders of magnitude	1–2	1–2	4–5	5–8	4–5	4–5
Speed for a element measurement (excluding sample) preparation)	Fast, 5–30 s (nebulization sample uptake 5–6 mL min^{-1})	Slow, 1–5 min, increases with matrix complexity	Intermediate, 1–2 min (nebulization sample uptak ~1 mL min^{-1})	Intermediate, 1–2 min (nebulization sample uptake ~1 mL min^{-1})	Minutes	Minutes to months (cooling is required)
Overall speed (including multi-element capability)	Slow, limited multi-element capability, 6 at most	Very slow, limited multi-element capability, 6 at most	Fast, multi-element capability	Fast, multi-element capability	Fast, multi-element capability	Fast, multi-element capability; slower if cooling is needed
Ease of use	Easy	Method development requires skilled user for complex matrices	Easy, common software skills are required	Intermediate, good software education is required	Easy	Easy
Interferences	Few	Well characterized, however, skill is required for correct interpretation and handling	Well characterized, spectral profiles must be taken for complex matrices	Well characterized, difficult to correct below 80 amu	Matrix dependency is high, matched standards are needed	Well characterized, some are eliminated by cooling

Table 12.1 *Continued*

Criterion	*FAAS*	*ETAAS*	*ICP-OES*	*ICP-MS*	*XRF*	*INAA*
Cost of instrument	Lowest,~40 K€	Intermediate, ~80 K€	High, ~100–200 K€	Very high, ~150–500 K€	Very high, ~100–500 K€	Very high, ~100–500 K€
Multi-element capability	Up to 6, mostly none	Up to 6, mostly none	Most systems are now simultaneous with multi-element capability	Yes	Yes	Yes
Capability of isotopic analysis	No	No	No	Yes	No	Yes, partially
Safety	Fire hazard, flammable gas, C_2H_2	Safe, inert gas Ar	Safe, inert gas Ar	Safe, inert gas Ar	Radiation hazards, precautions are required for X-ray protection	Radiation hazards, precautions are required for γ-ray protection

AFS was not included as its use is not as common as the other techniques in this table.

12.3 Evaluation of Individual Analytical Techniques

As we have discussed in the preceding paragraphs, a user has to consider several aspects in order to decide on the technique to be used. Personal and special needs and habits may play a role in selection. The so-called *advantages* or *disadvantages* may therefore become relative terms in many cases. In Table 12.1, the analytical techniques and relevant advantages and disadvantages are given.

CHAPTER 13

Essentiality and Toxicity of Some Trace Elements and Their Determination

13.1 Introduction

In this chapter, the functions of some trace elements will be summarized and recommended analytical methods will be given. Here we will discuss briefly only the so-called essential and probably essential trace elements and potentially toxic elements with some possibly essential function.

Recently, the World Health Organization published a book entitled *Trace Elements in Human Nutrition and Health* written by many experts in this field. Most of the information given in this chapter is taken from that book.[1]

For each element, their nutritional importance along with methods to determine them in food and diet by spectroscopic and nuclear techniques will be given. Sample introduction modes applied in atomic spectrometric techniques are given in Table 13.1. Wavelength data for AAS and ICP-OES are presented in Tables 13.2 and 13.3, respectively. The most used mass numbers for ICP-MS analysis are given in Table 13.4. The data shown in these tables cover the general applications, and some special and rare applications in the literature may not be included.

13.2 Essential and Probably Essential Trace Elements

Here we will discuss only boron, chromium, cobalt, copper, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc.

13.2.1 Boron

Little is known of the biochemical function of boron in human and animal tissues. Boron is distributed throughout the tissues and organs of animals and humans at concentrations mostly between 0.05 and 0.06 $\mu g(g \text{ fresh weights})^{-1}$, and several times these concentrations in bones.[2]

Table 13.1 *The most used sample introduction techniques for AAS, AFS, ICP-OES and ICP-MS. ETAAS application is valid for all elements but rarely for Hg*

	Nebulization	*Hydride generation*	*Cold vapour generation*
Al	X		
As	X	X	
B	X		
Cd	X		X
Co	X		
Cr	X		
Cu	X		
Fe	X		
Hg			X
Mn	X		
Mo	X		
Ni	X		
Pb	X	X	
Se	X	X	
Si	X		
Sn	X	X	
V	X		
Zn	X		

Table 13.2 *Commonly used analyte absorption lines for AAS*

Element	*Wavelengths of absorption*[a] *(nm)*					
Al	**309.27**	**309.28**	**396.15**	308.22	394.40	
As	**193.70**					
B	**249.70**	**249.80**	208.90			
Cd	**228.80**	326.10				
Co	**240.73**	242.49	241.16	252.14		
Cr	**357.87**	**359.35**	360.53	425.44	427.48	428.97
Cu	**324.75**	327.40	216.51	222.57	249.22	
Fe	**248.33**	248.82	252.29	271.90		
Hg	**253.65**					
Mn	**279.48**	279.83	403.08			
Mo	**313.26**	317.04	319.40			
Ni	**232.00**	231.10	341.48			
Pb	**217.00**	**283.31**	205.33			
Se	**196.03**	203.99	206.28			
Si	**251.61**	251.92	250.69	252.85		
Sn	**224.61**	286.33	235.48			
V	**313.84**	**318.40**	**318.54**			
Zn	**213.86**	307.59				

[a] Mostly used ones are in bold.

Consumption of foods of plant origin, and thus of boron, is often higher in countries with a lower incidence of osteoporosis. However, no comprehensive epidemiological studies establishing relationships between boron status and osteoporosis have

Table 13.3 *Commonly used analyte emission lines for ICP-OES*

Element	*Wavelengths of emission,*[a] *(nm)*								
Al	**308.215**	309.271	309.284	394.401	396.152				
As	188.977	**193.695**	197.198	228.812					
B	182.580	208.893	208.959	**249.678**	249.773				
Cd	**214.438**	226.502	228.802						
Co	**228.616**	236.379							
Cr	205.552	**206.149**	**267.716**	283.563	357.869				
Cu	213.598	219.958	224.700	324.700	**324.754**	327.396			
Fe	234.349	234.830	238.204	239.562	240.488	259.837	**259.940**	263.132	
Hg	184.888	**194.163**	253.652	296.728	312.567	365.010	404.660	546.070	576.960
I	178.215	182.976	206.163						
Mn	**257.610**	259.373	293.306						
Mo	**203.030**	**277.540**	281.615	287.150					
Ni	221.647	**231.604**	232.003	341.476					
Pb	216.999	**220.353**	224.689	280.199	283.306				
Se	**196.026**	203.985	206.279						
Si	212.412	250.690	**251.611**	288.158					
Sn	**189.926**	224.605	235.484	283.999	303.410				
V	292.401	309.311	**310.230**						
Zn	206.200	202.548	**213.856**	481.053					

[a] Mostly used ones are in bold

been conducted. Because the signs of chronic boron toxicity have not been clearly defined, it remains uncertain whether there are areas in the world where the population may be affected by it.

The daily intake of boron by humans can vary widely depending on the proportions of various food groups in the diet.[3] Foods of plant origin, especially fruits, leafy vegetables, nuts and legumes are rich sources. Wine, cider and beer are also high in boron. Meat, fish and dairy products are poor sources.

Some examples of boron supplied daily (in mg) by the various institutional menus in summer and winter, respectively, were: general diet, 1.58 and 1.15; low sodium diet, 1.46 and 0.47. Recent data suggested that boron intake from the "total diet" (as defined by the United States Food and Drug Administration) could be 1.52 ± 0.38 mg day^{-1}. Compared with the above values, the calculated average daily boron intake of English people is relatively high and variable, namely 2.8 ± 1.5 mg.[4]

Because the chronic toxicity of boron has rarely been studied or recognized, setting a threshold toxicity level is difficult. The richest food sources[2] of boron, such as nuts and dried fruits, generally supply 15–30 μg g^{-1}. An acceptable safe range of population mean intakes for boron for adults could well be 1.0–13 mg day^{-1}.

It is not easy to detect boron by normal INAA, because of the very short half-life of (n,γ) products. However, boron can be determined by the (n,α) reaction leading to an excited state of ^{7}Li. Either the α particles can be detected with nuclear emulsions or the γ rays from ^{7}Li can be measured. Prompt γ activation analysis (PGAA) is another nuclear method that can be used for determination of boron in food.

Boron forms very stable oxides, nitrides and carbides; its determination by FAAS is possible only for low-sensitivity application. Using ETAAS is also problematic due to carbide formation and rather low sensitivity as compared with other elements. ICP-OES is therefore used for low mg L^{-1} range and ICP-MS is the only choice for

Table 13.4 *Most abundant stable isotopes used in ICP-MS analysis*[a]

Element	*Isotope number*	*Mass (amu)*	*Abundance (%)*
Al	**27**	26.9815	100
As	**75**	74.9216	100
B	**11**	11.0093	80.1
	10	10.0129	19.9
Cd	114	113.9034	28.73
	112	111.9028	24.13
	113	112.9044	12.22
	111	110.9042	12.80
Co	**59**	58.9332	100
Cr	52	51.9405	83.79
	53	52.9407	9.50
Cu	**63**	62.9296	69.17
	65	64.9278	30.83
Fe	56	55.9349	91.72
	54	53.9396	5.8
	57	56.9354	2.1
	58	57.9333	0.28
Hg	**202**	201.9706	29.86
	200	199.9683	23.10
I	**127**	126.9045	100
Mn	**55**	54.9381	100
Mo	98	97.9054	24.13
	96	95.9047	16.68
	95	94.9058	15.92
Ni	58	57.9353	68.08
	60	59.9308	26.22
	64	63.9280	0.93
Pb	**208**	207.9766	52.4
	206	205.9744	24.1
	207	206.9759	22.1
	204	203.9730	1.4
Se	80	79.9165	49.61
	78	77.9173	23.78
	76	75.9192	9.36
	82	81.9167	8.73
	77	76.9199	7.63
	74	73.9225	0.89
Si	28	27.9769	92.23
	29	28.9765	4.67
	30	29.9738	3.10
Sn	120	119.9022	32.59
	118	117.9016	24.23
	116	115.9017	14.53
V	**51**	50.9440	99.75
	50	49.9472	0.25
Zn	64	63.9291	48.6
	66	65.9260	27.9
	68	67.9248	18.8

[a] Recommended mass numbers are in bold.

lower concentrations at μg L^{-1} level. Care is required as most glass materials contain boron and thus causes contaminations of this element.

13.2.2 Chromium

Chromium is an essential nutrient that potentiates insulin action and thus influences carbohydrate, lipid and protein metabolism. It probably works closely with the hormone insulin to help the cells take up glucose and break it down for energy.[5] Chromium is widely distributed in human tissues in extremely low and variable concentration. Most adult human tissues contain chromium levels of the order of 0.02–0.04 mg kg^{-1} on dry basis. The total content in the body of adult man is estimated to be less than 6 mg. Unlike most trace elements, tissue levels of chromium decline with age.[6]

Chromium concentrations in vegetable tissues range from 0.01 to 1 mg kg^{-1}, with the levels in most plants lying between 0.1 and 0.5 mg kg^{-1}. Countries in which refined food predominates in the diet are likely to have chromium deficiency since appreciable losses of chromium occur in the refining, especially in the process of sugar refining, so that white sugar contains very little chromium compared with the amounts in brown or raw sugar.[7]

The ^{50}Cr (n,γ)^{51}Cr reaction is used for the determination of Cr. The 320 keV γ ray emitted by 27.7 day ^{51}Cr is the most convenient for detection by neutron activation analysis. Usually, interferences to this γ ray are negligible in food samples.

Determination of total Cr is commonly performed by nebulizing solutions into flame or plasma; ETAAS applications are also used when low concentration levels are handled. Since radiation from D_2 sources is very weak at analyte line, 357.9 nm, Zeeman effect background correction is a better choice for ETAAS applications. Speciation studies for Cr(III) and Cr(VI) are often carried out using chemical separation systems prior to detection. In gaseous state, oxidation of Cr is rather easy; therefore a reducing air/C_2H_2 flame is used for FAAS. Use of N_2O/C_2H_2 flame is also possible and more interference-free as compared with the air/C_2H_2 flame. In addition, different sensitivities for Cr(III) and Cr(VI) are a problem for air/C_2H_2 flame while this is not the case for N_2O/C_2H_2 flame.

13.2.3 Cobalt

Cobalt is widely distributed throughout the body, without excessive accumulation in any particular organ or tissue. The highest concentrations generally occur in the liver, kidneys and bones. The total content of cobalt in the body of a normal 70-kg man has been reported to average 1.1 mg.[6] The primary function of cobalt is as a constituent of vitamin B_{12}. It also contributes to the formation of red blood cells. Cobalt is distributed widely among foods; hence it is readily available to man.[8] Most human diets provide cobalt in the range 15 to 60 μg day^{-1}.

The use of 1173.2 and 1332.5 keV γ rays coming from ^{60}Co is the best way to determine Co by INAA. These γ rays have no interferences. Since ^{60}Co has a long half-life (5.3 years), the γ rays can be counted 3–4 weeks or more after the end of irradiation.

Nebulization is used for sample introduction into flame and plasma. Normally, air/C_2H_2 flame is used for FAAS. Since the level of Co is rather low in food samples, ETAAS and ICP-MS are also widely used.

13.2.4 Copper

Copper is widely distributed in biological tissues, where it occurs largely in the form of organic complexes, many of which are metalloproteins and function as enzymes. Copper enzymes are involved in a variety of metabolic reactions, such as the utilization of oxygen during cell respiration and energy utilization.

In humans, acute copper poisoning is rare and usually results from contamination of foodstuffs or beverages by copper containers or from the accidental ingestion of copper salts. Symptoms of acute copper poisoning include salivation, nausea, vomiting and diarrhoea, all of which are probably due to the irritant effect of copper on the gastrointestinal mucosa.[9,10]

Copper is widely distributed in plants and animals. The copper content of vegetable crops is not greatly influenced by that of the soils on which they have grown but can increase markedly if leaf surfaces are contaminated by pollutants high in copper. Substantial species differences exist in the copper content of animal tissues used as food. Thus, ruminant liver and kidney can be high in copper. Good dietary sources of copper ($>2\ \mu g\ g^{-1}$) include seafood, organ meats, legumes and nuts. Refined cereals, sugar, milk and many other dairy products are low sources of the metal.[11,12]

Recent studies based on the analysis of duplicate diets suggest that the actual intake of adults may be in the range 1–1.5 mg day^{-1}.[13] In the light of the advantages conferred by improvements in methodology, it is probable that the data from these recent studies more accurately reflect typical adult intake of copper. Often overlooked, but nevertheless of potential significance, is the contribution that copper pipes can make to copper intake from drinking water. This can vary from < 0.1 mg day^{-1} in hard water areas to 10 times that level with some extremely acid soft waters.[6]

Several constituents occurring naturally in food have been found to affect the absorption of copper from the intestine and to increase or decrease its bioavailability. Apart from a low intake of dietary copper, which appears to increase the efficiency of copper absorption, the other main dietary factor, which enhances the bioavailability of copper, appears to be a high level of protein intake (100–150 g day^{-1}).[11]

A comparison of the proposed values for men (1.3 mg day^{-1}) and women (1.2 mg day^{-1}) with the data on copper intake from dietary surveys show that dietary copper supply is marginal or possibly inadequate in a number of countries. In only one of 10 recent, highly controlled IAEA studies were intakes below the basal minimum mean population copper intake. In contrast, a survey in the United Kingdom[13] suggests that the copper intake of at least 2.5% of the 2220 individuals investigated who were aged 16–65 years, was less than the estimate of individual mean copper requirements, given above for both men and women.

Short-lived 5.10 min ^{66}Cu ($E_\gamma = 1039.0$ keV) can be used in first short in neutron activation analysis for copper. The 1044.0 keV γ ray from 1.47 day ^{82}Br might interfere with copper determination.

Concentration of Cu in most food samples is sufficiently high to allow for determination by FAAS using an air/C_2H_2 flame. Solutions containing Cu can be ashed at temperatures as high as 1000 °C using ETAAS without any modifier. Nebulization is the choice for sample introduction also into plasma for ICP-OES and ICP-MS applications.

13.2.5 Iodine

The major role of iodine in nutrition arises from the important role played by the thyroid hormones in the growth and development of humans and animals. The effects of iodine deficiency on growth and development are seen at all stages of development, and particularly in the foetus, the neonate and the infant, *i.e.* in periods of rapid growth. The term "goitre" has been used for many years to describe the effect of iodine deficiency.[14] Iodine administration in the form of iodized salt, bread or oil has been demonstrated to be effective in the prevention of goitre in adults. It may also reduce existing goitre in adults; this is particularly true of iodized oil injections.[15]

The iodine contents of foods and of total diets differ appreciably and are influenced by geochemical, soil and cultural conditions that modify the iodine uptake by staple crops and foods of animal origin. Marine fishes and shellfishes have the highest iodine content, 830 and 800 mg (kg fresh bases)$^{-1}$, respectively. Eggs, meat, milk and cereal grains have iodine in the range of 50–90 mg kg^{-1}. Cooking reduces the iodine content of food. Data show that frying reduces the iodine content by 20%, grilling by 23% and boiling by as much as 58%.[16]

The usual recommended level for the population mean intake of iodine is 100–150 mg day^{-1}. This level is adequate to maintain the normal thyroid function that is essential for normal growth and development. In the presence of goitrogens in the diet, the intake should be increased to 200–300 mg day^{-1}.[17]

Iodine can be determined by $^{127}I(n,\gamma)^{128}I$ reaction. ^{128}I, which has a half-life of 25 min emitting 450 keV γ rays, is used often by INAA without any chemical separation.

Determination of I using AAS or ICP-OES is not commonly used. ICP-MS provides sufficient sensitivity for most food and diet samples; direct nebulization is used.

13.2.6 Iron

There is a total of about 4 g of iron in the body. About 60–70% of this iron is found in haemoglobin and the related myoglobin. Iron is also required for the activity of certain enzymes involved in energy production and about 10% of the body pool of iron is used in this way. The remaining 20–30% of iron is stored in the liver as a complex salt called ferritin.[18]

Vitamin B_{12}, ascorbic acid and copper also play an important role in the effective utilization of iron, and consequently have an influence on the daily requirement. Good dietary sources of iron are meat, which on average provides 25% of the intake (especially liver and kidney), egg yolk, bread, flour and other cereal products, potatoes and vegetables. Iron is found in cereals, but due to refining, 50% may be lost. Some natural drinking water contains quite high levels of iron; if water is stored in iron tanks or passes through cats-iron pipes, objectionable concentrations of iron

may be reached.[8,18] The recommended daily allowance is 10–12 mg of iron for male and 15 mg of iron for female. Deficiency of iron is seen in many countries, mostly due to low meat and meat product consumption.

The ^{58}Fe (n,γ)^{59}Fe reaction is used for the determination of Fe in INAA. The 45.1 day ^{59}Fe emits γ ray at 1099 and 1290 keV.

In food and diet samples, concentration of Fe is sufficiently high so that FAAS with an air/C_2H_2 flame can be used. Sample introduction into flame and plasma is performed by nebulization. Speciation of Fe(II) and Fe(III) is an often needed but a rather delicate reaction since the concentration of these ionic species show a dependency on pH.

13.2.7 Manganese

Manganese has been shown to be an essential element in every animal species studied. Manganese is found in the liver, skin, bones, kidney and muscles of animals. The human body has about 12 to 20 mg manganese in a 70-kg man. This quantity is only one-fifth of the estimated total of copper and 1/100th of that of zinc.[6]

Whole grains and cereal products are the richest dietary sources of manganese, and fruits and vegetables are somewhat less. Dairy products, meat, fish and poultry are poor sources. Tea is a rich source of manganese, but typical drinking water consumed at the rate of 2 L daily contributes only about 40 to 64 μg or about 2 to 3% of the amount furnished by diet. A provisional daily dietary manganese intake of 2.0 to 5.0 mg is recommended for adults.[19]

Neutron activation is a very good method for the determination of manganese. Irradiation with thermal neutron yields ^{56}Mn (2.56 h), with 846 keV γ ray.

Determination of manganese by FAAS requires an air/C_2H_2 flame. ETAAS is also widely used for low-level applications. Nebulization is the only practicable technique for sample introduction into flame or plasma for AAS, ICP-OES and ICP-MS analysis.

13.2.8 Molybdenum

Discovery of molybdenum in the enzyme xanthine dehydrogenaseoxidase involved in the conversion of tissue purines into uric acid provided the first evidence of the essentiality of this element. It acts as a co-factor for certain enzymes, but the reactions catalysed by these enzymes may not really be vital to human cells. Molybdenum is important in nutrition primarily because of the danger of toxicity to grazing animals; plants grown on soils high in this mineral can accumulate such high levels that they poison the animals feeding upon them.[20]

Estimates of the daily intake of molybdenum differ widely. This may be attributed partly to the technical difficulties of estimating dietary molybdenum accurately. However, extreme regional variations occur both in the soil availability of molybdenum and in the extent to which this is reflected by its content in food crops. Therefore, tabulations of expected molybdenum concentrations in various foods are of limited value. The foods that contribute the most to the molybdenum intake are milk, beans, breads and cereals. Legumes, cereal grains, some dark green vegetables,

liver and kidney are good sources of the mineral. The provisional recommended range for the dietary intake of molybdenum based on average reported intakes is set at 75 to 250 μg day^{-1} for adults and older children.[19,21]

Animal products, with the exception of liver, are generally poor sources of molybdenum, whereas vegetables, especially those grown on neutral or alkaline soils, are rich in the element. In contrast, vegetables grown on leached acid soils may be molybdenum-deficient. Vegetation grown on soils derived from shales, mineralized granites and some peats tend to have an elevated molybdenum content, which may give rise to molybdenum toxicity in farm livestock and possibly to threshold molybdenum toxicity in humans. Industrial pollution has resulted in high molybdenum contents of crops in the environment of molybdenum-processing plants.[22]

Short-lived ^{101}Mo (13.6 min) and long-lived ^{99}Mo (66 h) are formed by (n,γ) reactions with molybdenum. The former has the higher sensitivity, but the latter is more suitable, as its longer half-life makes chemical separation easier, if necessary.

Determination of molybdenum using FAAS and either an air/C_2H_2 flame or a N_2O/C_2H_2 flame for slightly better sensitivity is possible. Since this element forms stable oxides and carbides, its determination is problematic in AAS. ETAAS technique for Mo also suffers from tailing and memory effects due to carbide formation. Ar plasma has a much higher temperature and a rather inert environment so that ICP-OES and ICP-MS techniques are easily applied for this element. Nebulization is used for sample introduction into flame or plasma. Abundance in Earth's crust and thus in food samples is very low; ICP-MS would be the choice of many researchers for analysis.

13.2.9 Nickel

The four typical nickel-containing enzymes found in plants and microorganisms, namely urease, hydrogenase, methylcoenzyme M reductase and carbon monoxide dehydrogenase, have been comprehensively described in a number of reviews.[23,24] Evidence that nickel functions similarly in animals or humans is awaited with interest. If the nickel deficiency is severe, growth and haematopoiesis are depressed, especially in animals with a marginal iron status or in methyl-depleted animals.

Serum concentrations above 1.0 μg of nickel L^{-1} probably indicate a chronically excessive intake of nickel. However, high serum nickel can also reflect a recent intake of a large dose of highly available nickel.

Total dietary nickel intakes of humans vary greatly with the amounts and proportions of food of animal (low-nickel) or plant (high-nickel) origin consumed, and with the amount of refined or processed foods in the diet. Approximately half the total daily intake of nickel is usually derived from the consumption of bread, cereals and beverages. Recent reports indicate that diets often provide less than 150 μg of nickel daily.[25,26]

Available information indicates that most monogastric animals have a nickel requirement of less than 200 μg per kg of diet.[27] Thus, if animal data are extrapolated to humans, it is reasonable to suggest a basal nickel requirement of less than 100 μg daily for adults. The finding that an oral dose as low as 600 μg nickel as nickel sulfate given with water to fasting subjects produced a positive skin reaction

in some nickel-sensitive individuals suggests that the threshold level for toxicity can be quite low in specific situations, and could thus be set at less than 600 μg day^{-1}.

The 2.56 h ^{65}Ni is the most convenient way to determine Ni in food by INAA. The 1120 and 1500 keV γ rays can be used.

Nickel is present in food or diet samples at very low concentrations. Air/C_2H_2 flame is exclusively the choice for FAAS, but the sensitivity is seldom sufficient for most of the cases. ETAAS is widely used as well as ICP-OES and ICP-MS. Using ICP-OES does not offer any significant advantage over FAAS. Sample introduction is performed by nebulization into flame and plasma.

13.2.10 Selenium

Until recently, the only known metabolic role for selenium in mammals was as a component of the enzyme glutathione peroxidase which, together with vitamin E, catalase and super oxide dismutase, is a component of one of the antioxidant defence systems of the body.[28]

Selenium occurs in all the cells and tissues of the body in concentrations that vary with the tissue and with the level of selenium in the diet. Dietary selenium intakes are difficult to estimate because of the variation in the selenium content of locally produced and consumed foods which are determined by the selenium content of the soil in which food is grown.[19] Selenium functions as part of an antioxidant enzyme and can substitute for vitamin E in some of that vitamin's antioxidant activities.[29] Selenium is necessary for growth and fertility in animals and for the prevention of various diseases, which shows variable responses to vitamin E.[30]

Seafood, kidney, liver and to a lesser extent other meats are consistently good sources of selenium, whereas grains and other seeds are more variable, depending on the selenium content of the soils in which they are grown. Fruits and vegetables generally contain little selenium. Drinking water usually makes only a small contribution to selenium intake.

Recommended dietary allowance for adult man is set at 70 μg day^{-1}. The allowance for adult women is set at 55 μg day^{-1}. The recommended allowance for children is 20–30 μg day^{-1}.

Keshan disease is a selenium-responsive endemic cardiomyopathy that mainly affects children and women of childbearing age in certain areas of China. This disease was considered a "water and soil" disease because residents of the Se areas felt that some component of the local water or soil caused it. The fact that domestic animals raised in these areas suffered from white muscle disease suggested that selenium could be lacking in the environment, and indeed soil samples from Keshan disease areas were found to be low in selenium.[31]

The possibility that increased intakes of selenium might protect against the development of cancer in humans has generated great interest. However, a number of epidemiological studies have now been reported which show no relationship between selenium status and cancer risk[32]. Moreover, a recent analysis of the relationship between selenium and cancer suggests, "the question of whether selenium protects against cancer is still wide open".[33]

Typical values of the selenium content of various foods have been reported as follows (μg per kg wet weight): liver, kidney and seafood, 0.4–1.5; muscle meats, 0.1–0.4; cereals and cereal products, $<0.1->0.8$; dairy products, $<0.1-0.3$; and fruits and vegetables, <0.1 (12). Apart from these natural differences in the selenium content of foods, the amount of selenium ingested in the diet is a function of the food habits of the individual (*i.e*. consumption patterns) and particularly of the geographical origin of the food (*i.e*. whether it comes from regions with selenium-rich or selenium-poor soils). Thus the contribution of various food groups to the overall dietary intake of selenium can differ markedly from one country to another.

Food consumption patterns would also affect the proportion of dietary selenium that is bioavailable. If most of the dietary selenium is derived from cereals, its bioavailability will be higher than if it is derived from certain fish, such as tuna.

The distribution of intakes was skewed upwards by a few relatively high selenium levels (median = 74 μg day^{-1}). Mutanen *et al*.[34] found a wide dispersion of individual selenium intakes, since the mean values based on daily diet composites of 40 Finnish men were 53 ± 32 and 50 ± 20 μg day^{-1} in September and December 1981, respectively; the distribution of selenium contents also showed a slight upwards skew. As part of a year-long mineral balance study of North Americans, Levander and Morris[35] analysed four 7-day food composites collected during the various seasons of the year from 12 men and 15 women ranging in age from 19 to 50 years. The mean dietary selenium intake calculated on the basis of an average of the four weekly composites was 90 ± 14 and 74 ± 12 μg day^{-1} for men and women, respectively.

Because of the scarcity of human data, the first dietary standard for selenium was based on the extrapolation of the results of studies on animals.[19] It was noted that a diet with a selenium content of 100 μg kg^{-1} was sufficient for maximal growth and reproductive performance in all mammalian species examined. If it is assumed that adult humans consume 500 g of a mixed diet daily (dry basis), such selenium content would result in a selenium intake of 50 μg day^{-1}. This figure was adopted as the lower limit of the Estimated Safe and Adequate Daily Dietary Intake proposed in 1980 by the United States National Research Council.[19] To allow for the possible influence of other dietary factors on selenium metabolism and in order to take into account the effect of individual variation on requirement, selenium in the range of 50–200 μg day^{-1} was suggested as safe and adequate for adults, with correspondingly lower levels for infants and children.

No recommendation was made for pregnant or lactating women. In an attempt to pinpoint more closely the human requirement for selenium, several balance studies were carried out in various countries.[29] When the results were compared internationally, however, it soon became apparent that balance techniques for the determination of mineral requirements were particularly inappropriate for selenium because of the marked ability of individuals to adapt and maintain balance at many levels of selenium intake by modifying the faecal and urinary excretion of the element in line with changes in selenium status.

Biochemical techniques for the early detection of pathological effects arising from over-exposure to dietary selenium should be developed. Data are needed from which

acceptable upper limits for selenium intake can be defined for infants, children, adolescents and pregnant or lactating women.

Both $^{76}Se\ (n,\gamma)^{77m}Se$ and $^{74}Se(n,\gamma)^{75}Se$ reactions are used for the determination of Se in food by INAA. The 20 s ^{77m}Se, which emits 162 keV γ ray, is becoming a very fast and sensitive way to determine Se in food and diet. The 127 day ^{75}Se is also used commonly for Se determination.

Although an air/C_2H_2 flame can be used, FAAS is not suitable for most applications due to insufficient sensitivity. Both ETAAS and HGAAS are used frequently. For total Se determination, all the Se species must be converted into one form, usually Se(VI) using modifiers and a suitable pyrolysis step. On the other hand, only Se(IV) provides a good signal for HGAAS; conversion of other species into this form must be accomplished in sample solutions prior to analysis. HG-AFS is also occasionally used. For plasma systems, sample introduction by both nebulization and hydride generation is used. Speciation studies for this element is most popular and important; separation by chromatography or electrophoresis is needed prior to atomic detection.

13.2.11 Silicon

Silicon, the second most abundant element in the Earth's crust, is not found free in nature, but occurs chiefly as oxide and silicates. Asbestos, tremolite, the feldspars, clays and micas are but a few of the silicate minerals.

The essential function of silicon has been independently demonstrated by two groups of researchers in two species of experimental animals.[36,37] Growth stimulation of rats following administration of silicon was observed only when low-silicon (5 μg of silicon per g of diet) synthetic rations based on crystalline amino acids were fortified with 250–500 μg of silicon per g of diet. However, regardless of dietary composition, all other experiments in which silicon deficiency has been induced have demonstrated the importance of the element for the normal development of connective tissue and bone in chickens and rats.

The general manifestations of silicon toxicity are collectively described as silicosis. As with other essential elements, certain chemical forms of silicon are toxic if inhaled or ingested in large amounts. The carcinogenic effects of asbestos fibres have caused serious public health problems where some forms of asbestos have been used extensively in construction projects in the past.[38]

Foods of plant origin contain more silicon than those of animal origin. Whole grasses and cereals may contain 3–6% silica. The silicon intake of adults in Finland,[39] the United Kingdom and the USA[40] varies between 21 and 49 mg day^{-1}. Fruits and most animal products contribute little silicon, whereas most foods of vegetable origin contain the element in amounts roughly proportional to their fibre content.[38] The factors governing the biological availability of silicon have not been adequately defined.

While silicon has been shown to play an essential role in the development of bone in two species of experimental animal, for which a requirement of 100–250 mg per kg of diet has been suggested, no data are available from which human requirements for silicon can yet be estimated.

Neutron activation analysis for silicon is based on the $^{30}Si(n,\gamma)^{31}Si$ reaction. The radionuclide formed ($t_{1/2} = 2.62$ h) emits β radiation and a small amount (0.07%) of γ radiation with energy of 1260 keV. Because of the low yield of γ rays and low cross-section of the reaction, it is not easy to determine Si by INAA. For trace amounts of silicon, a chemical separation is needed before the β or γ radiation is measured.

Silicon forms stable oxides and carbides. Oxide formation in an N_2O/C_2H_2 flame is minimized, but the sensitivity is not sufficient for food analysis. ETAAS is used but care and use of modifiers are required to control carbide formation. Nebulization is used for flame and plasma for sample introduction. ICP-OES is significantly superior to FAAS; ICP-MS has the best sensitivity among all the atomic techniques.

13.2.12 Tin

Originally the presence of tin in tissue was attributed to environmental contamination; however, careful and detailed studies by Schwarz *et al*.[41] demonstrated that tin produced an acceleration of growth in rats and further met the standards for an essential trace element. As a member of the fourth main chemical group of elements, tin has many chemical and physical properties similar to those of carbon, silica, germanium and lead.

Biological interest in tin initially focussed on its toxic potential to man through the contact of foods with tin-coated cans and tinfoil. Tin has now been shown to be an essential nutrient for the growth of rats. Schwarz *et al*.[41] found the growth rate to be enhanced by nearly 60% if 1–2 mg per kg Sn was added as stannic sulfate to a highly purified diet fed in a plastic isolator environment**.** Nielsen[42] states that the only evidence that supports essentiality of tin is that a dietary supplement of tin, both in the inorganic or organic form, improved the growth of sub-optimally growing, apparently riboflavin-deficient rats but did not result in optimal growth**.**

Most fresh and frozen foods probably contain less than 1 μg of tin g^{-1}. The major source of tin is canned foods. Usually, the dietary intake of tin is 1.0–2.3 mg per kg of diet.[43,44]

Determination of Sn by FAAS is possible by using both air/C_2H_2 flame and N_2O /C_2H_2 flames, but the method is not sensitive for food analysis. Both ETAAS and HGAAS using a heated quartz tube atomizer are used for higher sensitivity; the former technique is known to have interference problems in heavy matrices. Speciation analysis for Sn is popular, especially in seafood. For sample introduction into plasma, both nebulization and hydride generation are used for determination of Sn by ICP-OES and ICP-MS.

13.2.13 Vanadium

The discovery of vanadium-activated enzymes in lower forms of life lends credence to the view that vanadium has similar roles in higher animals. Vanadium-dependent enzymes in lower organisms include nitrogenase in bacteria,[45] which reduces molecular nitrogen to ammonia.

Anke and co-workers[46] reported some reasonably well-substantiated deficiency signs in goats which, when fed only 10 ng of vanadium per g of diet, as opposed to 2 μg of vanadium per g of diet, exhibited a higher abortion rate and produced less milk during the first 56 days of lactation. Toxic effects resulting from the intake of large amounts of vanadium in the diet are unlikely. Toxicity usually occurs only as the result of industrial exposure to high levels of airborne vanadium. Signs of excessive vanadium intake in humans include gastrointestinal disturbances and green tongue.[47,48]

Serum vanadium may be a good indicator of exposure to high dietary vanadium. Cornelis *et al.*[49] found that human serum vanadium was in the range 0.016–0.939 ng mL^{-1}, most values being below 0.15 ng mL^{-1}. Thus, serum vanadium values above 1.0 ng mL^{-1} probably indicate excessive exposure.

The V^{5+} ion is absorbed 3–5 times as effectively as V^{4+}. Thus, the effect of other dietary components on the form of the vanadium present in the stomach, and the speed with which it is transformed into V^{4+}, probably markedly affect the percentage of ingested vanadium absorbed. This is supported by the finding that a number of substances, including EDTA, ascorbic acid, chromium, protein, ferrous iron, chloride and aluminium hydroxide,[50] can reduce vanadium toxicity.

The body of a normal adult man has been estimated to contain 10–25 mg of vanadium, most of which is present in the bones, teeth and fat. A diet high in unsaturated fatty acids from vegetable sources results in higher vanadium intake than one containing saturated fats from animal sources. Large concentrations of vanadium up to 43 mg kg^{-1} were found in soyabean oil and in corn, olive, linseed and peanut oils. Beef, pork, deer and chicken fats were relatively rich in this element.

The daily dietary intake of vanadium is of the order of a few tens of μg and may vary widely.[51] A Total Diet Study in the United Kingdom in 10 diet samples show that it supplies an average of 13 μg of vanadium per day.[52] Estimation of the daily intakes of vanadium for eight age–sex groups suggested a range of 6.2–18.3 μg in the United States Food and Drug Administration's Total Diet Study.[53] Myron *et al.*[54] found that beverages, fats and oils, and fresh fruits and vegetables contained the least vanadium, ranging from 1 to 5 ng g^{-1}. Whole grains, seafood, meats and dairy products generally contained 5–30 ng vanadium per g. Byrne and Kosta[51] obtained similar results. Only a few food items, including spinach, parsley, mushrooms and oysters, contain relatively large amounts of vanadium.

The $^{51}V(n,\gamma)^{52}V$ reaction is used for determining of V in food. The 3.76 min ^{52}V emits γ rays at 1440 keV.

Vanadium forms fairly stable oxides and not very stable carbides. Therefore its determination by ETAAS in carbon atomizers is feasible. A N_2O/C_2H_2 flame is commonly used; sensitivity is not sufficient for low levels encountered in food samples. ICP-OES and ICP-MS determinations are usually preferred. Nebulization is used for sample introduction into flame or plasma.

13.2.14 Zinc

Most biochemical roles of zinc reflect its involvement in a large number of enzymes or as a stabilizer of the molecular structure of subcellular constituents and mem-

branes. Zinc participates in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids; it has shown to play an essential role in polynucleotide transcription and translation and thus in the processes of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all forms of life.

Zinc is generally found in the body in the liver, muscle, bones, skin and blood. It has very important function in the reaction of certain enzymes.[55] The whole body of a 70-kg man is estimated to contain 1.4–2.3 g of zinc.[56]

Good sources of zinc include red meat, liver, kidneys, whole grain cereals, eggs, shellfish, nuts and cheese.[57,58] Zinc content of fruits, vegetables and refined foods is low. Zinc absorption is better from animal sources than plant sources owing to phytic acid.[58]

An evaluation of the most reliable balance studies indicates that at least 12 mg of zinc in a mixed US diet is required to maintain the existing zinc status of a healthy young man. The recommended allowance for men is set at 15 mg day^{-1}. The allowance for women, owing to of their lower body weight is set at 12 mg day^{-1}.[19]

The principal clinical features of severe zinc deficiency in humans are growth retardation, a delay in sexual and skeletal maturation, the development of artificial and acral dermatitis, diarrhoea, alopecia, a failure of appetite and the appearance of behavioural changes.[59] An increased susceptibility to infections reflects the development of defects in the immune system. Not only the range of food items selected, but also the degree of refinement of any constitutent cereals influences the zinc content of the total diet. Fats, from which zinc is virtually absent, tend to dilute zinc from the total diet.

As the primary goal of nutrition in developing countries is to provide sufficient energy, the most appropriate basis for the comparison of foods is the relationship of their zinc content to their energy content. Lean, red meat is an outstanding zinc source. Furthermore, its zinc is present in a highly available form. Many staple foods provide amounts of zinc similar to those of foods derived from animal tissues. However, energy sources such as fats, oils, sugar and alcohol have very low zinc content. Green leafy vegetables and fruits are only modest sources of zinc (as of energy) because of their high water content.

The daily intake of zinc in industrialized countries from diets characteristically high in fat, refined sugar and animal protein has been reported to be approximately 10–12 mg or 1.0–1.2 mg zinc per MJ (mega joule). However, a recent survey of over 2100 adults in the United Kingdom suggested a mean zinc intake of 9.7 ± 3.3 mg day^{-1}.[13]

Both $^{68}Zn(n,\gamma)^{69m}Zn$ and $^{64}Zn(n,\gamma)^{65}Zn$ reactions are used for the determination of Zn by INAA. But 1115.5 keV γ ray coming from 245 day ^{65}Zn is the most convenient one.

Zinc is one of the few elements that can be easily determined using an air/C_2H_2 flame by AAS; among the other analyte elements determined by FAAS, Zn gives the most sensitive results. Nebulization is used for all flame and plasma techniques. ETAAS determination is also rather easy and free of problems except the contamination of samples. Contamination from glass and plastic containers as well as environment, such as dust particles in air, is very common; care must be exercised in selection and cleaning of container materials, tubing, pipette tips and other materials that are in contact with sample solutions. Although ICP-MS is the most sensitive

technique, many analyses can be safely and accurately performed by low-cost FAAS. ICP-OES determination is not significantly more sensitive than FAAS.

13.3 Potentially Toxic Elements: Some Possibly with Essential Function

Here we will discuss only arsenic, fluorine, cadmium, lead, mercury and aluminium.

13.3.1 Arsenic

Arsenic occurs in the trivalent and pentavalent forms in foods, water and the environment, and is widely distributed geologically as a component of about 245 different minerals. Industrial production is approximately 50 000 tons $year^{-1}$; the main uses are in agricultural chemicals, such as pesticides, herbicides, cotton desiccant, and wood preservatives, and as additives to animal foods, as well as in pharmaceutical products, all of which have a direct impact on the environment.[60] Arsenic is one of those elements that is both essential and toxic. Inorganic species are more toxic; for most common species an order of toxicity may be given as As(III) > As(V) > monomethylarsonate (MMA) > dimethylarsinate (DMA) > arsenobetaine (AsB). Most of species present in fish and other sea products are rather non-toxic species such as AsB, arsenocholine and As-containing sugars.

Although arsenic compounds are best known historically for their toxicity, their pharmacological action is also well documented. Less well documented is the increasing evidence of the essential function fulfilled by very low dietary arsenic intake in four species of experimental animals. The biological effects of arsenic depend markedly on the chemical form in which the element is presented, inorganic compounds being more toxic than most organic ones. Most living organisms convert the former by methylation into a large variety of less toxic organoarsenic compounds, which are then excreted. These compounds contribute substantial amounts of arsenic to human diets containing fish and other seafood.

The major cause of concern in connection with arsenic is the potential toxicity of its compounds to humans. Consumption of water containing 0.8 mg of arsenic per L over extended periods of time and a dietary intake of approximately 3 mg of arsenic per day for 2–3 weeks have been identified as causes of arsenic intoxication. However, the toxicity of arsenic compounds depends so greatly on their chemical nature that general estimates of safe intakes cannot be made with confidence.

The beneficial effects of substantially lower intakes of arsenic have been demonstrated in three independent studies involving four animal species offered basal diets providing less than 1 μg of arsenic per g. Most foods and feeds of terrestrial origin contain less than 7 μg of arsenic per g dry weight[61]; the levels present in those of marine origin are substantially higher, ranging up to 80 μg g^{-1}. Dietary intake is therefore greatly influenced by the amount of seafood in the diet. Based on recent surveys in several countries, the daily arsenic intake of adults is estimated to be <200 μg, and often below 100 μg day^{-1}.[61] It is unlikely that the arsenic intake from uncontaminated

diets poses a risk of toxicity. Extrapolation from animal experiments suggests that human adult intake in the range 12–25 μg day^{-1} is probably adequate to meet any possible requirement.[2]

Because inorganic arsenic is known to be carcinogenic in humans, there is understandable concern to limit human exposure to excessive environmental concentrations of the element. However, the metabolism and effects of arsenic can differ markedly, depending on the chemical nature of the arsenic source. These differences partly account for the provisional nature of the recommended safe exposure limit for adults of 15 μg per kg of body weight per week.[62] Since experimental arsenic deficiency has been produced in four species, the element may have an essential function. If a human requirement for arsenic does exist, it is probably close to 20 μg day^{-1} for adults and is easily met by most diets.

The $^{75}As(n,\gamma)^{76}As$ reaction can be used for determining of As. The 1.096 day ^{76}As emit 657.2 and 559.5 keV γ rays. It is recommended to count these γ rays during first long count, 1–2 days after the end of irradiation. The 559.5 keV γ ray is usually free from other interferences except 554.5 keV γ ray from 1.47 day ^{82}Br. Since half-lives of these two isotopes are not very different and the amount of As is far less than the Br in food, one has to make careful separation of these γ rays.

Determination of As by FAAS is possible by using a lean air/C_2H_2 flame; however, only the samples containing As in medium–high mg L^{-1} level can be analysed. Since As concentration is usually much lower in food samples, FAAS has no practical importance. ETAAS and HGAAS using a heated quartz tube atomizer are the widely used techniques for low level As samples. Determination of As by HG-AFS is also possible. Hydride generation technique has higher sensitivity as compared with nebulization for ICP-OES and ICP-MS; however, depending on the level of As content, nebulization can also be used for these techniques.

Speciation of As is an increasingly important issue; a chromatographic or electrophoretic separation can be used prior to detection by one of the atomic spectrometric techniques.

13.3.2 Fluorine

Fluorine in the form of fluoride is well distributed in nature and enters the body as a variable constituent of both drinking water and food. The total intake of adults is usually within the range of 0.2–2.0 mg of fluoride per day but higher intakes are not uncommon where fluoride content of drinking water is high.

Fluoride is reported to be required for the transformation of osteocalcium phosphate to apatite, the chief mineral component of skeletal tissue. Higher fluoride contents increase the crystallinity of apatite and decrease its solubility in acid. Although the exact biological roles of fluoride in humans have not been established, animal experiments have suggested that it may be a structurally important constituent of bone collagen and of the glycosaminoglycans of the vascular system, the skin and other tissues.[63]

Ingested fluoride accumulates in bone tissue. The fluoride content of human bones varies from 300 to 7000 μg per g of dry tissue depending on the total fluoride exposure. Studies carried out in India suggest that the body fluoride burden of individuals

exposed to higher levels of environmental fluoride may be 2–3 times higher than normal, judging from the fluoride content of bone dry matter. Bone fluoride is a good indicator of lifetime exposure of the body to the element.

There is significant individual variability in the metabolic handling of fluoride.[64] Retention is also influenced by diet. Millet-based diets promoted significantly greater retention of fluoride than rice-based diets in normal, healthy and active populations of young adults, given 11 mg of elemental fluorine as sodium fluoride.[65]

Ingestion of large amounts of fluoride (5–40 mg day^{-1}) in drinking water produces severe forms of skeletal deformity. These include kyphosis, fixed spine and other joint deformities, and the dramatic skeletal manifestation of the disease genu valgum. Endemic genu valgum has been reported from fluorosis areas in India, Kenya, the United Republic of Tanzania and other African countries.[66, 67]

In assessments of intakes of fluoride and their relation to tolerable levels, account should be taken of fluoride ingestion through water and food, as a result of environmental contamination from both natural and industrial sources. Enormous variation exists in water consumption pattern both in the same individual and from one individual to another. Age, occupation, environmental temperature, perspiration and food habits all determine water intake, and consequently may markedly influence fluoride intake.

Although fluoride should probably be regarded as essential, there is no evidence so far from human studies that overt clinical signs of fluoride deficiency exist. No specifically diagnostic clinical or biochemical parameters have been related to fluoride inadequacy. The Expert Consultation was therefore unable to specify a minimum desirable intake. However, in view of the toxicity associated with excessive fluoride ingestion from a variety of sources, recommendations for maximum safe intakes are required. For this purpose, dental mottling may be taken as a definitive indication of toxicity.

Food samples can be activated with thermal neutrons in a reactor in a short irradiation, usually 5–10 s. The 1634 keV γ ray coming from the 10.7 s ^{20}F can be measured. Fluorine can also be determined based on reactions with either charged particles, (p,αn), (α,n), (p,pn), or fast neutrons (n,p), (n,2n); however, this use is not very common.

Determination of fluorine by atomic spectrometric techniques is not feasible and thus is not used. In aqueous medium, fluoride ion is determined usually by either using an ion-selective fluoride electrode and potentiometry or an anion column and ion chromatography.

13.3.3 Cadmium

Evidence of a progressive accumulation of cadmium in the kidney with increasing age suggests that cadmium exposure will adversely affect health, particularly in older age groups. However, cadmium absorption is also higher in children than in adults.[68]

Local surface distribution of mine wastes from mineral-rich deposits often increases cadmium levels in crops. Soil distribution of urban wastes and sludges is

also responsible for significant increases in the normally low ($<0.15\ \mu g\ g^{-1}$) cadmium content of most food crops.

Cereals and other vegetables normally account for about 50% of cadmium intake which, in children, is typically in the range 2–25 $\mu g\ day^{-1}$ and in adults 10–50 $\mu g\ day^{-1}$. The highest values within these ranges were all reported from countries noted for extensive industrial activities involving the processing of cadmium-rich ores or other raw materials. The influence of high-cadmium environmental anomalies on the dietary intake of cadmium is substantially increased if the diet contains such food items.

The Joint FA/WHO Expert Committee on Food Additives[69,70] recommends that 7 μg of cadmium per kg of body weight should be regarded, provisionally, as the maximum tolerable weekly intake of cadmium. For a 65-kg man, this corresponds to a dietary intake of 65 $\mu g\ day^{-1}$. A survey of dietary intakes of cadmium in two countries indicated that adult intakes varied from 0.9 to 7 $\mu g\ kg^{-1}$ per week; the corresponding range for infants or children from 10 countries was 1.9–9.9 $\mu g\ kg^{-1}$ per week.[71] Representative mean intakes of cadmium were in the range 10–60 $\mu g\ day^{-1}$ for adults and 5–20 $\mu g\ day^{-1}$ for children. Concentrations of cadmium in most foods are typically less than 0.15 $mg\ kg^{-1}$. Notable exceptions are shellfish and kidneys, the former containing typically 1–2 mg of cadmium per kg and the latter 0.5 $mg\ kg^{-1}$. The cadmium content of vegetable and cereal crops is influenced by the soils in which they are grown if these are polluted by cadmium-rich industrial or urban wastes or are derived from geological parent materials containing the zinc–cadmium ore, sphalerite. WHO has set the upper guideline value of 3 $\mu g\ L^{-1}$ for cadmium in drinking water.[72]

Three independent investigations reported during the period 1976–1977 are occasionally cited as evidence that cadmium at low concentrations in diet or drinking water may be required to maximize the growth of experimental animals. The growth of rats maintained on semi-synthetic diets increased by up to 8% as dietary cadmium was increased from 12.5 to 50 or 100 $\mu g\ kg^{-1}$.[73] A brief report of the study has been reported in which the growth of goats before and during pregnancy was markedly restricted when dietary cadmium was held below 20 $\mu g\ g^{-1}$ rather than 250 μg per kg feed.[74] Further evidence, accompanied by adequate statistical verification of the data, is needed before cadmium can be regarded as physiologically essential.

Either ^{115}Cd (53 h) or ^{111m}Cd (48.6 min) can be used for determining cadmium. Both radionuclides are induced through (n,γ) reaction. The radionuclide ^{115}Cd is more advantageous in activation analysis because of its longer half-life. Since the amount of Cd is rather small in food, in general it might need radiochemical separation of its longer half-life. If the latter is used, a rapid radiochemical separation is needed.

Cadmium atoms are relatively easily formed, a lean air/C_2H_2 flame is thus most commonly used for FAAS. Regarding ETAAS applications, ashing temperatures are limited to about 800 °C. Although nebulization into flame and plasma is the most widely used way for sample introduction, CVAAS is also possible; application of this technique for low-level samples is becoming increasingly more popular. Another rather novel alternative, also rarely used, is CVAFS.

13.3.4 Lead

Lead is widely distributed in rocks and soils, the highest concentrations being found in localities in which it has accumulated by geochemical mineralization and particularly where lead-rich ores have been mined and the waste has been dispersed on land used for cultivation or grazing. Other methods of industrial or urban waste disposal and the presence of road traffic can also increase oil lead and, since its phytoxicity is low, such high lead concentration, as often, remain unsuspected.

Bread, cereals and beverages account typically for about 35% of the daily intake of lead, the remainder being derived from a variety of food types. Mean dietary intakes of children have been reported to be in the range 9–278 μg of lead per day and for adults 20–282 μg day^{-1}. A typically high dietary intake (e.g. >500 μg of lead per day) was found in one Indian investigation.

Lead does not have any suitable isotope for neutron activation analysis. Lead is easily atomized and determined by using FAAS with an air/C_2H_2 flame. Its concentration, however, is very low in most food samples so that sensitivity of FAAS is often not sufficient. ETAAS is widely used; employing modifiers and platform atomization can minimize non-spectral interferences. In order to minimize background absorption problems, longer but less sensitive line at 283.3 nm is used instead of the most sensitive line at 217.0 nm. Generally nebulization is used for sample introduction into flame and plasma; hydride generation is also used with an heated quartz atomizer for AAS or plasma sources for ICP-OES or ICP-MS applications.

13.3.5 Mercury

Methylmercury readily crosses the placenta into the foetus and passes into the neonate via maternal milk. It is readily incorporated into the central nervous system at early stages of development. Epidemiological evidence from instances of accidental exposure to methylmercury indicates that perinatal exposure is particularly harmful to the developing infant and presents a substantially greater hazard than exposure in adulthood. Studies on experimental animals also show that inorganic (mercuric) mercury is absorbed and retained in the brain more readily during perinatal periods of exposure.[75]

Biological concentration in specific food types also markedly influences the dietary intake of mercury and in addition, may enhance its toxicity. Mercury derived from natural sources is probably greater than that from industrial emissions by a factor of at least 10, most being in the form of inorganic compounds. Typical total dietary intakes reported were in the range 2–6 ug of mercury per day for children and 2–140 ug day^{-1} for adults, the mercury of fish and foods of marine origin accounting for about 40% and 20% in fruit and vegetables. The contribution from fish is markedly greater in some communities and this, coupled with its bitransformation to toxic organic mercury in marine ecosystems, increases the significance of environmental anomalies in mercury distribution irrespective of their precise causes.

Many of the factors influencing the significance of the diet as a source of lead, cadmium and mercury are considered in more detail elsewhere.[76] The Joint

FAO/WHO Expert Committee on Food Additives[77,78] provisionally recommends that total mercury intake should not exceed 5 μg per kg of body weight per week with not more than 3.3 μg per kg per week as methylmercury. Of 26 national dietary surveys recently reviewed by IAEA,[79] none showed mean dietary intakes approaching this figure. Other sources report mean intakes of mercury from diet and water of 0.2–3.1 μg per kg of body weight per week for infants and 0.5–2.0 μg per kg for adults.[71] Typically, 1% of the total intake of inorganic mercury is derived from drinking water and 84% from the diet; fish can account for between 20 and 85% of total mercury, mostly as methylmercury.

With the exception of the accidental consumption of cereal grains dressed with organomercurials, the potential threat posed by terrestrial pollution with mercury compounds is appreciably less than that arising from the alkylation of mercury by aquatic components of the food chain. Mercury contamination of the marine environment is rapidly reflected by increased intakes of readily absorbable methylmercury derived from the tissues of predatory fish. Such considerations make difficult the toxicological interpretation of data on dietary mercury contents if evidence of its form is not available.

Many stable mercury isotopes can serve as targets for activation with neutrons. Of the activities induced, those of ^{203}Hg (47 days, $E_\gamma = 279$ keV) and of ^{197m}Hg(23 h, $E_\gamma = 77$ keV) are of most importance. It was demonstrated that the use of 77 keV γ ray would give more sensitive and faster results for the determination of Hg in food and environmental samples.

CVAAS is the most common way for Hg determinations at low concentrations. Since this element is very volatile, ETAAS is almost never used, as ashing without losing analyte is not normally feasible. Use of cold vapour generation may also be coupled with AFS resulting in very sensitive applications. Cold vapour generation is also widely applied for ICP-OES and ICP-MS.

Organic species are more toxic. Mercury speciation applications are most common using a chromatographic or electrophoretic separation step prior to determination by any of the techniques described above. Food and diet samples have very low levels of Hg; therefore the most sensitive determination techniques are always needed.

13.3.6 Aluminium

Aluminium is among the most plentiful elements in the Earth's crust, accounting for 8% of the total. Human exposure to aluminium may have increased since both the solubility and the bioavailability to plants and aquatic life of environmental aluminium may have been increased by acid rain and industrial emissions. Aluminium is an extremely versatile metal with a wide variety of uses, *e.g.* in packing and building materials, paint pigments, insulating materials, abrasives, cosmetics, food additives and antacids. This results in a wide range of human contacts with the metal and a consequent potential impact on human populations. Factors influencing exposure to aluminium and its tolerance by human subjects have been extensively reviewed by the Joint FAO WHO Expert Committee on Food Additives.[80]

There is no substantiated evidence that aluminium has any essential function, in animals or humans. The main concern with respect to aluminium and health is its

potential toxicity if exposure is excessive. Dialysis encephalopathy in a large number of patients with renal failure undergoing chronic dialysis was shown by Alfrey[81] to be attributable to the high aluminium content of some water used for the preparation of dialysates. Excess aluminium also affects the skeleton by markedly reducing bone formation, resulting in osteomalacia. Such problems have practically disappeared since the use of aluminium-free deionized water for dialysis became routine.[81]

Aluminium interacts with a number of other elements, including calcium, fluorine, iron, magnesium, phosphorus and strontium, and, when ingested in excess, can reduce their absorption. Because of this property, it has been used therapeutically to treat fluorosis and to reduce phosphorus absorption in uraemic patients. These interactions are unlikely to present health risks at typical dietary intakes, estimated at 3–14 mg day^{-1}. Water would add from less than 100 μg to 1 mg L^{-1}, depending on its pH, and the use of aluminium cooking utensils with acidic foods might increase intake.[82]

It has been provisionally suggested that the tolerable weekly intake of aluminium may be approximately 7 mg per kg of body weight.[80] Milk, milk products and cereal products account, typically, for about 60% of the total dietary intake of aluminium.[83] Mean intakes in the range 3–14 mg of aluminium per day have been reported.

In conclusion, there is no known risk to healthy people from typical dietary intakes of aluminium. Risks arise only from the habitual consumption of gram quantities of aluminium antacids over long periods of time; they are markedly increased in persons with impaired kidney function. Long-term intravenous application always results in serious toxicity.

Aluminium can be determined by the reaction $^{27}Al(n,\gamma)^{28}Al$ with thermal neutrons to yield the 2.8 min ^{28}Al, which emit γ rays at 1780 keV. Since food and diet contain phosphorous in much higher concentration than Al, the $^{31}P(n,\alpha)^{28}Al$ reaction would also yield ^{28}Al which interferes determination of Al. This could be avoided by reducing the fast neutron flux.

Aluminium is determined widely by atomic spectrometric techniques. FAAS applications require a fuel-rich N_2O/C_2H_2 flame. ETAAS is also used often when higher sensitivity is needed. Sample contamination is a problem especially at low concentrations due to high abundance of this analyte and hence care must be exercised. Nebulization is the common way of sample introduction into flame and plasma.

13.4 Literature on Determination of Trace Elements in Food Samples

The field of publication regarding trace element determinations in food is very active. Annual reviews published in *Journal of Analytical Atomic Spectrometry*, entitled as "Atomic spectrometry update. Clinical and biological materials, foods and beverages" are very useful. A collection of selected literature of recent years is given in Table 13.5; each item on this table contains brief information on the technique employed, sample handled and some notes regarding the nature and content of the study presented.

Table 13.5 *Literature for determination of elements in food samples*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Al	Al, Cr	Baby foods	ETAAS	Slurry sampling, aqueous calibration	84
Al		Beer	ICP-OES	Hot acid digestion; storage conditions affected Al content	85
Al	Al, Cd, Cu	Beer, beer ingredients	ETAAS	Suspension sampling for solid samples	86
Al		Beers	ETAAS	Comparison of aluminium and glass containers	87
Al		Canned soft drinks	ETAAS	Can lining defects	88
Al	Al, Ca, Cl, Fe, K, Mg, N, P, Si	Chicken dung, dates	INAA	Milk SRM supplied by IAEA	89
Al	Al, Cr, Mn, Mo	Cow's milk, human milk, infant formulas	ETAAS	Slurry sampling	90
Al		Drinking water, tap water	ETAAS	Fractionation by filtration and ion exchange	91
Al		Fast foods	ETAAS	Ingredients, packaging and intakes	92
Al		Fish	ETAAS	MW-activated O_2 plasma for sample pretreatment	93
Al	Al, Ba, Si, Sr, Ti	Food	ICP-OES	Direct fusion using lithium metaborate	94
Al		Food	ETAAS	Migration from packaging and utensils	95
Al		Food	ICP-OES	Effect of aluminium packaging	96
Al		Food	FAAS	Acid digestion; extraction; oxine complex formation	97
Al		Food	ETAAS	Adult intake, India	98
Al		Food, beverages	ETAAS	Use of HNO_3–V_2O_5 as oxidizing agent; slurry sampling; Spanish diet	99
Al	Al, Pb	Fruit juice, milk	FAAS	Preconcentrated on activated carbon-cupferron	100
Al		Fruit juice, soft drinks, water	ETAAS	Digestion using HNO_3–V_2O_5	101
Al		Herbs, spices	ETAAS	Use of HNO_3–V_2O_5 as oxidizing agent; slurry sampling; Spanish diet	102
Al	Al, Zn	Infant formula, infant food	ETAAS	MWD	103
Al		Infant formulas	ETAAS	Samples from Spain	104
Al		Juices	ETAAS	FI, slurry sampling	105
Al		Meat	ETAAS	Herbs and spices as main sources	106

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Al		Milk powder	ETAAS, XRF	Slurry sampling and chemical modifiers	107
Al		Parenteral solution	ICP-OES	Preconcentration by cloud-point method; FI	108
Al		Parenteral solution	ICP-OES	Raw materials and product contamination	109
Al		Potable water	ICP-MS	On-site fractionation using chelating columns	110
Al		Seafood, meat	ICP-OES	Lyophilization, HNO_3–HF, MWD	111
Al		Tea	ICP-OES	Sephadex column separation	112
Al		Tea	Several	Review	113
Al	Al, Ba, Mg, Mn	Tea leaf	ICP-OES	Slurry nebulization	114
Al		Wine	ETAAS	Chemical modifiers were compared	115
Al	Al, Cu, Fe, K, Na, Pb	Wine	ETAAS	Suitability of ultrafiltration for fractionation study	116
Al		Wine	ETAAS	Simple method, wide applicability	117
Al		Wine	ETAAS	Solid-phase extraction on Amberlite XAD-1180	118
Al		Wine, beer, alcoholic beverages	ETAAS	Influence on packaging on contamination	119
As		Animal feed additives	ICP-MS, HPLC	Speciation	120
As		Apple	ICP-MS	Speciation using HPLC	121
As		Baby foods	ETAAS	Slurry atomization	122
As		Baby foods	ICP-MS	Speciation, HPLC; enzymatic digestion using trypsin and pancreatin	123
As	As, Sb	Baby foods, fish	HGAAS, ETAAS	Speciation, HPLC	124
As		Beer wort	HG-AFS	Speciation for As(III) and As(V) based on different sensitivities in HG-AFS	125
As		Beer, wine	HG-AFS	FI-HG-AFS; no mineralization was needed	126
As		Breast milk, water	HGAAS	As exposure and content in milk were studied	127
As	As, Cd, Hg	Calcium supplements	ETAAS	Samples from Korea	128
As		Carrots	ICP-MS	Accelerated solvent extraction, speciation, HPLC	129
As		Carrots	ICP-MS	Effect of contamination on growing location; speciation, HPLC	130
As	As, Se	Coffee	HG-ICP-OES	MWD using HNO_3 and H_2O_2	131

As	As, Sb	Cow's milk	HG-AFS	MWD, FI	132
As	As, Cd, Hg, Pb	Dietary supplements	ICP-MS	High-resolution ICP-MS	133
As	As, Br, I	Drinking water	ICP-MS	Ion chromatography for speciation	134
As		Drinking water	ETAAS	Inorganic As speciation	135
As		Drinking water	ICP-MS	Speciation using IEC; comparison of Tris and phosphate buffers	136
As		Drinking water	HG-ICP-MS	Speciation, CE	137
As		Drinking water	ICP-MS	EDTA treatment to stabilize As(III) and As(V) in Fe-rich waters	138
As	As, Sb	Drinking water	HG-AFS	Speciation	139
As		Drinking water	HGAAS	Online reduction to As(III); FI	140
As		Duplicate diets, body fluids	HGAAS	Intake study by the adult population of Bombay city	141
As		Edible mushrooms	ICP-MS	Speciation, HPLC; extraction by focussed MWD	142
As		Edible oils	ETAAS	Dissolution in *n*-heptane; Zeeman effect background correction	143
As		Edible seaweed, urine	ICP-MS, ES-MS	Samples from South China, speciation, HPLC	144
As	As, Se	Egg powder, mussel tissue, nuts	ICP-MS, ETAAS, SICP-OES, HGAA	Comparison of analytical techniques and digestion procedures	145
As	As, Se	Fish	ICP-MS, HPLC	Speciation using HPLC-ICP-MS	146
As	As, Cd, Hg, Pb	Fish	ETAAS	Samples from Adriatic Sea	147
As		Fish	ICP-MS, HPLC	Extraction by MW and using MeOH–water mixture; speciation using HPLC-ICP-MS, ion-exchange and ion-pair LC	148
As		Fish	ICP-MS, HG-AFS	Samples from North Sea	149
As		Fish, shellfish	ICP-MS	Speciation, HPLC	150
As		Fish, tap water	FAFS	Ultrasonic nebulization; arsenobetaine, arsenocholine	151
As	As, Se, Sb, Te	Fish, water SRMs	ICP-MS	Ion-exchange HPLC for speciation	152
As	As, Se	Food	HGAAS	CF, FI, cryogenic trapping	153
As		Food	HGAAS	FI, interferences were masked by ascorbic acid–KI in HCl	154
As	As, Cd, Pb	Food colours	ETAAS	Ni as chemical modifier	155
As		Food composites	ICP-MS	Lyophilization, extraction by sonication; speciation, IEC	156

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
As		Food SRMs	ETAAS	Optimum conditions	157
As	As, Se	Food	HGAAS	MWD, dry ashing	158
As	As, Cd, Hg, Pb	Food	Various	Inter-laboratory study	159
As	As, Cd, Hg, Pb	Food	ICP-MS, FAAS	Samples from Catalonia, Spain	160
As		Food	AAS	Dietary intakes, duplicate portion sampling, samples from Belgium	161
As		Food	ICP-MS	Samples from West Bengal, contamination study	162
As		Food	HGAAS	Digestion using HNO_3–H_2O_2, transforming to As(III) by KI	163
As		Food	HG-AFS	Speciation, ion-pair chromatography; effect of food treatment processes	164
As		Human milk	HGAAS	Samples from German mothers, study on WW II contamination effects	165
As		Human milk, cow's milk	AA	Samples from Turkey	166
As		Liquorice confectionary	HGAAS	Throat pearl samples	167
As		Marine tissues	ICP-MS	Speciation using HPLC; extraction from 165freeze-dried tissues	168
As	As, Cd, Hg, Pb	Medicinal plants	ICP-MS	Samples from Spain	169
As	As, Bi, Sb, Se, Te	Milk	HG-AFS	Slurry sampling	170
As	As, Cd, Pb	Milk powder	AAS, UV-VIS	Secondary reference material	171
As	As, Sb	Milk powder	HG-AFS	Sonication in aqua regia; speciation	172
As	As, Se	Milk SRM	ICP-MS	Tertiary amine digestion; polyatomic 168interference study	173
As		Mineral water	ICP-MS, CE	Speciation	174
As		Mung beans	ICP-MS	Mung beans as bioindicator or environmental 173contamination	175
As		Mushrooms	FAAS, ETAAS, 175ICP-MS	Speciation by anion-exchange column and HPLC; arsenite, DMA, arsenate, MMA were determined	176

As		Mushrooms	ICP-MS, INAA	Speciation, HPLC, ion-exchange chromatography; bioaccumulation	177
As		Mushrooms	HG-AFS	HPLC, photo-oxidation, HG, cryogenic trapping, AFS	178
As		Orange juice	HGAAS	Dry ashing	179
As		Oyster	HG-AFS	Speciation, HPLC. Extraction using CH_3OH–H_2O. MW heating	180
As		Oyster	ES-MS, HG-AFS	Comparison of techniques; HPLC for arsenosugar analysis	181
As		Oyster	ICP-MS	MWD, speciation by ion-exchange HPLC	182
As		Peanut butter	ICP-MS	Speciation, IC	183
As		Rice	ICP-OES, ICP-MS	Grinding and MWD; samples from Italy	184
As		Rice	ICP-MS	Speciation, HPLC; loss of stability by grinding	185
As		Rice	ICP-MS	Speciation	186
As		Rice straw	HG-AFS	Speciation, HPLC; comparison of extraction methods	187
As	As, Cd, Pb	Seafood	ICP-OES, ICP-MS	A single MWD technique for a variety of sea food samples	188
As	As, Hg, Se, Zn	Seafood	INAA	Samples from Malaysia	189
As		Seafood	HGAAS	Methods for pretreatment were compared	190
As		Seafood	Various	Review; As and its species in seafood	191
As		Seafood	ETAAS	Sample preparation methods were compared	192
As		Seafood	HG-AFS	Speciation using cationic column	193
As		Seafood	HGAAS	MW-assisted distillation, speciation	194
As		Seafood	HGAAS	Speciation using HPLC, MWD	195
As		Seafood	HGAAS	Speciation, HPLC; arsenobetaine	196
As		Seafood	HGAAS, HG-AFS	Speciation, cation- and anion-exchange columns, online thermo-oxidation	197
As		Seafood	ICP-MS	Samples from China, speciation, HPLC	198
As		Seafood	ETAAS	MWD, inter-laboratory study	199
As		Seafood	ICP-MS, ES-MS	Speciation of arsenosugars using HPLC	200
As		Seafood	HGAAS	Speciation, HPLC; arsenobetaine migration in brine, tinned products	201
As		Seafood	AAS??	MWD	202

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
As		Seafood, water	HG-AFS	Speciation, HPLC and online thermo-oxidation	203
As		Seaweed food products	FAB-MS	Arsenosugars; characterization	204
As		Sperm whale	ICP-MS	Speciation	205
As	As, Cu, Fe, Pb	Sugar	HGAAS	Decomposition techniques were compared	206
As		Sugar	ETAAS	Carbon was removed using oxygen at pyrolysis step, Pd was used as chemical modifier	207 208
As		Sugar	ETAAS	Comparison of chemical modifiers	
As	As, Hg	Sugar beet	HGAAS, CVAAS	Method development	209
As	As, Hg	Sunflower oil	ETAAS	Extracting of species using 0.1 M NH_3–0.01 M EDTA	210
As		Tap water	ETAAS	Preconcentration of arsines in a liquid drop containing AgDDC	211
As		Traditional Chinese medicines	HG-AFS	Thiourea to reduce As(V) to As(III) and to reduce interferences	212
As		Tuna fish	ICP-MS, HG-ICP-MS	Speciation using a single column	213
As	As, Pb	Vegetables	ICP-OES, ICP-MS	Samples from Bangladesh; contamination study	214
As		Vegetables	HGAAS	Uptake study from vegetables in Chile	215
As	As, Cu, Mn, Sb, Se	Water	ETAAS	Simultaneous detection and Zeeman background correction	216
As	As, Se	Water	ICP-MS	Speciation, HPLC	217
As	As, Se	Water	HG-ICP-MS	Speciation, CE; online reduction	218
As		Water	HGAAS	MW-assisted oxidation	219
As		Water	ICP-MS	Speciation, HPLC	220
As		Water	HG-ICP-MS,	High-resolution ICP-MS and HG-ICP-MS were compared	221
As		Water	ICP-MS	Speciation using HPLC	222
As		Water, CRMs	ETAAS	FI; preconcentration by complexation; speciation	223
As		Water, scalp hair	EDXRF	Samples from Bangladesh; contamination study	224
As	As, Se	Water, urine, kelp RMs	HG-ICP-MS	Pneumatic nebulization and hydride introduction; speciation	225

As		Well water	ICP-MS	Speciation in samples from West Bengal; HPLC	226
As		Wheat flour	ETAAS	Preconcentration by precipitation as Ag salt; $Pd(NO_3)_2$ as matrix modifier	227
As	As, Cd, Pb	Wine	ICP-MS	Samples from Canary Isles	228
As		Wine	ICP-MS, FI	FI-ICP-MS	229
As		Wine	HGAAS	Evaporation of ethanol as sample preparation; inorganic and total arsenic	230
As		Wine, kelp powder	ICP-MS	Speciation using reverse-phase HPLC; arsenite, DMA, arsenate, MMA were determined	231
B	B, Ba, Ce, Cu, Mg, Pb, Sr, Zn	Apple, carrot	ICP-MS, HPLC	SEC-ICP-MS was used for speciation	232
B		Coffee, tea	ICP-OES	MWD or hot-water extraction; In as internal standard	233
B		Hazelnuts	ICP-OES	Samples from Turkey; natural source of B	234
B		Radish roots	ICP-MS	Speciation, SEC	235
B		Plants, plant-derived foods	ICP-OES	Ultrasonic nebulization	236
Cd	Al, Cd, Cu	Beer, beer ingredients	ETAAS	Suspension sampling for solid samples	86
Cd	As, Cd, Hg	Calcium supplements	ETAAS	Samples from Korea	128
Cd	As, Cd, Hg, Pb	Dietary supplements	ICP-MS	High-resolution ICP-MS	133
Cd	As, Cd, Hg, Pb	Fish	ETAAS	Samples from Adriatic Sea	147
Cd	As, Cd, Pb	Food colours	ETAAS	$NH_4H_2PO_4$ as chemical modifier	155
Cd	As, Cd, Hg, Pb	Food	Various	Inter-laboratory study	159
Cd	As, Cd, Hg, Pb	Food	ICP-MS, FAAS	Samples from Catalonia, Spain	160
Cd	As, Cd, Hg, Pb	Medicinal plants	ICP-MS	Samples from Spain	169
Cd	As, Cd, Pb	Milk powder	AAS, UV-VIS	Secondary reference material	171
Cd	As, Cd, Pb	Seafood	ICP-OES, ICP-MS	A single MWD technique for a variety of seafood samples	188
Cd	As, Cd, Pb	Wine	ICP-MS	Samples from Canary Isles	228
Cd	Cd, Cu	Artificial food digests	ICP-MS	ID, inter-laboratory comparison; RM certification studies	237
Cd	Cd, Pb, Se	Baby food	ETAAS	Slurry sampling	238
Cd		Baby foods	ETAAS	Contamination study	239
Cd		Beer	ETAAS	German beer; ecological and conventional plant growing	240

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Cd	Cd, Pb	Beer, carbonated drinks, juice, wine	ETAAS	Samples from Finland	241
Cd	Cd, Zn	Bovine offal, muscle	ETAAS	Low levels in samples from organically reared	242
Cd		Brassicas	PIXE	Cd uptake and elemental distribution in Cd-stressed plants	243
Cd	Cd, Pb	Breast milk	ETAAS	MWD	244
Cd	Cd, Pb, Zn	Carrot, endive	FAAS, ETAAS	Samples from Slovenia, effect of power plant	245
Cd	Cd, Pb	Cereals	ICP-MS	Japanese food samples and intake study	246
Cd	Cd, Pb	Cereals, pulses	ICP-MS	Northeastern Chinese products were evaluated	247
Cd	Cd, Hg	Chinese herbal medicines	HG-AFS	Intermittent flow system; non-dispersive AFS	248
Cd	Cd, Pb	Cocoa	ICP-MS	Fractionation using SEC	249
Cd	Cd, Pb	Cocoa	ICP-MS	Bioavailability; *in vitro* gastrointestinal enzymolysis procedure	250
Cd	Cd, Ni, Pb, V	Cucumber plants	TXRF	Uptake and transport processes were studied	251
Cd	Cd, Pb	Diet	ETAAS, ICP-MS	Two techniques are compared; 418 diet homogenate samples	252
Cd	Cd, Hg, Pb	Diets	FAAS	Samples from Poland, duplicate diet technique	253
Cd	Cd, Cu, Pb	Drinking water	W-coil AAS	Simultaneous determination by AAS	254
Cd		Drinking water	ICP-OES	Preconcentration on a minicolumn	255
Cd	Cd, Cu, Ni, Pb	Drinking water	FAAS	Preconcentration on activated carbon column	256
Cd	Cd, Pb	Duplicate diet, food	ETAAS	Samples from Korea	257
Cd	Cd, Cu, Pb, Zn	Durum and soft wheat	ETAAS, FAAS	Italian wheat samples	258
Cd		Durum wheat	ICP-OES	Genetic variation for Cd concentration	259
Cd		Equine meat	ETAAS	Cd intake study	260
Cd	Cd, Pb	Fish	ETAAS	Samples consumed in Finland	261
Cd		Flour	ICP-MS	Ingestion–uptake study	262
Cd		Flour	FAAS	Preconcentration by atom trapping	263
Cd	Cd, Pb	Food	ICP-MS	Environmental exposure study, Japan	264
Cd		Food	ICP-MS	Cd levels in Japanese; blood, urine and food analyses	265

Cd		Food	ETAAS	Modified L'vov furnace; solid sampling	266
Cd	Cd, Pb	Food CRMs	ETAAS	High-pressure ashing and MWD were compared	267
Cd	Cd, Cr, Pb	Food packaging	ICP-MS	Paper and paper board analysis; MWD	268
Cd	Cd, Pb	Food SRMs	ICP-MS, HGAAS, CVAAS	High-pressure ashing and MWD were compared	269
Cd	Cd, Pb	Food	ETAAS	Cryogenic grinding and slurry sampling, Rh–W-coated graphite tubes	270
Cd	Cd, Cr, Ni, Pb	Food	ICP-MS	Online dilution for food digests	271
Cd	Ca, Cd, Ce, Fe, Pb, Zn	Food	ICP-MS	1991 UK survey on diet; quality control in multi-element analysis	272
Cd	Cd, Pb	Food	FAAS	Beam injection flame furnace AAS, slurry sampling	273
Cd		Food	AAS	Duplicate diet study on German adults and children	274
Cd		Food	FAAS	Atom trapping for preconcentration	275
Cd		Food	ETAAS	Market basket samples, 1988–1991	276
Cd		Food, beverages	FAAS, ETAAS	Samples from Japan	277
Cd	Cd, Ni, Pb	Food, tissues	ETAAS	MWD	278
Cd	Cd, Pb	Foodstuffs	ETAAS	Multi-element AAS	279
Cd	Cd, Pb	Foodstuffs	ETAAS	Duplicate diet study; samples from Korea	280
Cd	Cd, Pb	Fruit juice	ETAAS	Home-made ETAA spectrometer	281
Cd	Ca, Cd, Cu, Mg, Pb, Zn	Goat's milk	ETAAS	Samples from Czech Republic	282
Cd		Honey	FAAS	Column preconcentration by complex formation	283
Cd	Cd, Cu, Se, Zn	Human milk	AAS	Fortification in fertilizers and effect on maternal milk.	284
Cd	Cd, Pb	Infant cereals	ETAAS	Dry ashing and acid dissolution before injection	285
Cd		Infant formulas	ETAAS	Differences related to products	286
Cd		Japanese foods	ETAAS, FAAS	Determination in 519 samples of foods and beverages	287
Cd	Cd, Ni, Pb	Liver, kidney, food	ETAAS	MWD; chemical modifiers	288
Cd	Cd, Pb	Lupin seeds	ETAAS	Australian lupin seeds	289
Cd		Manchego cheese	ETAAS	Levels during manufacturing and ripening	290
Cd	Cd, Pb	Medicinal plants	AAS	Samples from India	291
Cd	Cd, Cr, Pb, Zn	Milk powder	ICP-MS	Ultrasonic slurry sampling; ETV and reaction cell	292

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Cd	Ca, Cd, Cu, K, Mg, Na, P, Zn	Mother milk	FAAS, ETAAS, ICP-OES	*Postpartum* samples; influence of Cd on essential elements	293
Cd	Cd, Mn, Ni	Mushrooms	ICP-OES	Treated compost, uptake measurements	294
Cd		Mushrooms	FAAS	Role of *Boletus edulis* in transport and storage	295
Cd	Cd, Pb	Mussels	ETAAS	Extraction from tissue using dilute HNO_3 and ultrasound	296
Cd		Mussels	FAAS	Precipitation of Cd as an ion pair, online pre-concentration	297
Cd		Mussels	FAAS	FI preconcentration on minicolumn using complexation	298
Cd	Cd, Pb	Mustard plants	ICP-OES	Uptake from soil	299
Cd	B, Ca, Cl, Cd, H, K, Na, S	Orange juice	INAA	Neutron capture prompt γ ray activation analysis	300
Cd		Peanut products	ETAAS	Samples from Australia	301
Cd		Porcine and bovine kidney	ETAAS, FAAS	MWD, variations in Cd concentration in porcine and bovine kidneys; effect of freezing samples	302
Cd		Porcine tissue	ETAAS	Cd in pigs fed with organic and conventional feed	303
Cd	Cd, Cu, Pb	Potable water, plant material	FAAS	Preconcentration by column	304
Cd		Radish, spinach	ETAAS	Cadmium-containing protein characterization	305
Cd	Cd, Cu, Pb	Rice	ICP-MS	ID, international measurement evaluation programme	306
Cd		Rice	ETAAS	Seventeen countries	307
Cd		Rice	ICP-MS	Milling effect on loss of analyte	308
Cd		Rice	FAAS	FI, preconcentration by precipitation	309
Cd	Cd, Cr, Fe, Pb	Rice flour	ICP-MS, ID	ID-ICP-MS; cool plasma	310
Cd		Rice flour	ICP-MS	Direct determination by LA	311
Cd		Seafood	ETAAS	Slurry sampling	312
Cd	Cd, Pb	Sunflower oil	ETAAS	Digestion using HNO_3–V_2O_5; 21 samples	313
Cd		Total diet	ETAAS	Dietary intake study, Belgium	314
Cd		Traditional Canadian foods	FAAS, ETAAS	Cd exposure, animal organs	315

Cd	Cd, Pb, Zn	Vegetable oil	ICP-MS	ETV-ICP-MS; emulsion sampling	316
Cd		Vegetables	FAAS	Water-cooled steel atom trap, derivative measurement	317
Cd	Cd, Pb	Vegetables, protein foodstuff	ETAAS	MWD, Pd as MM	318
Cd	Cd, Pb	Water	ETAAS	Batch preconcentration, slurry sampling	319
Cd		Water, tea infusion	ETAAS	Generation of volatile analyte species; preconcentration in graphite furnace	320
Cd	13-C, Cd, 15-N, Pb, Se, Sr	Wheat	ICP-MS	Identification of geographic origin	321
Cd		Wheat grain	ETAAS	Bran, whole meal, flour, white flour	322
Cd	Cd, Cu, Pb, Se	Wheat, wheat products, corn bran, rice flour	ETAAS	MWD procedures were compared	323
Cd	Cd, Pb	Wine	ETAAS	Transverse heating and Zeeman effect background correction; Pd and Mg as chemical modifiers	324
Cd		Wine	ICP-OES	Column preconcentration	325
Cd	Cd, Pb	Wines	ETAAS	MWD in concentrated nitric acid; samples from Korea	326
Cd		Wines	ETAAS	Performance of chemical modifiers were compared	327
Co		Flour fractions	ETAAS	Online column preconcentration, FI, effect of milling process	328
Co	Co, Cu, Zn	Milk, vitamin samples	FAAS	Batch and column preconcentration	329
Co	Co, Cr, Ni	Preserved foods	ETAAS	Platform	330
Co	Co, Cr, Ni	Vegetables	ETAAS	Sample preparation methods were compared; slurry sampling	331
Co		Water	ETAAS	Knotted reactor for preconcentration of Co-nitroso-R salt complex	332
Co	Co, Cr, Ni	Wheat flour	ETAAS	Slurry sampling	333
Cr	Al, Cr	Baby foods	ETAAS	Slurry sampling, aqueous calibration	84
Cr	Al, Cr, Mn, Mo	Cow's milk, human milk, infant formulas	ETAAS	Slurry sampling	90
Cr	Cd, Cr, Pb	Food packaging	ICP-MS	Paper and paper board analysis; MWD	268
Cr	Cd, Cr, Ni, Pb	Food	ICP-MS	Online dilution for food digests	271
Cr	Cd, Cr, Pb, Zn	Milk powder	ICP-MS	Ultrasonic slurry sampling; ETV and reaction cell	292

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Cr	Cd, Cr, Fe, Pb	Rice flour	ICP-MS, ID	ID-ICP-MS; cool plasma	310
Cr	Co, Cr, Ni	Preserved foods	ETAAS	Platform	330
Cr	Co, Cr, Ni	Vegetables	ETAAS	Sample preparation methods were compared; slurry sampling	331
Cr	Co, Cr, Ni	Wheat flour	ETAAS	Slurry sampling	333
Cr		Breakfast cereals	ETAAS	Acid digestion; rich source for Cr	334
Cr	Cr, Ni	Cooking utensils	ETAAS	Cooking utensils, Cr, Ni leaching from stainless steel pans	335
Cr	Cr, Pb	Drinking water	ETAAS	Preconcentration in electrokinetic flow system	336
Cr		Drinking water	ETAAS	Simultaneous separation and preconcentration for Cr(III) and Cr(VI) species	337
Cr		Feeds	ETAAS	Samples from Spain	338
Cr		Food CRMs	ICP-MS, ETAAS	Comparison of techniques; USN for ICP-MS	339
Cr	Cr, Ni	Food, food utensils	ETAAS	Leaching of Cr and Ni from cooking utensils was studies.	340
Cr	Cr, Mn	Food	ETAAS	Overnight digestion	341
Cr		Food	ETAAS	Samples from Greece; meat, fish, cereals, pulses	342
Cr		Foodstuffs	FAAS	Preconcentration by solvent extraction	343
Cr		Herbs, spices	ETAAS	Digestion by HNO_3–V_2O_5	344
Cr		Infant milk formulas	ETAAS	Cr(VI) determination, separation by anion-exchange column	345
Cr		Medicinal herbs	XRF	Removal of low atomic number elements	346
Cr		Milk powder, mussels	ETAAS	W-coil ETA; various chemical modifiers were compared	347
Cr		Milk, sugar	ETAAS	Fast, direct method, Zeeman background correction	348
Cr		Parenteral nutrition	FAAS	Preconcentration by complexation	349
Cr		UHT milk	ETAAS	Cr(VI) by protein precipitation, then elution by HNO_3	350
Cr		Water	ICP-MS	Speciation of Cr(III) and Cr(VI); ion chromatography	351

Cr		Water	FAAS	Cr(III) and Cr(VI) were separated on a C-18 column; speciation analysis	352
Cr		Water	ETAAS	Speciation for Cr(III) and Cr(VI); column preconcentration	353
Cr		Water	FAES	Speciation; online preconcentration	354
Cr		Water, dairy products	ETAAS	Speciation using a ZnO column	355
Cr	Cr, Ni	Wheat flour	ETAAS	Slurry sampling	356
Cr		Wine	ETAAS	No sample pretreatment for wine, mineralization for other alcoholic drinks	357
Cr		Wine, grapes	ETAAS	Effect of mineralization; samples from France	358
Cu	Al, Cd, Cu	Beer, beer ingredients	ETAAS	Suspension sampling for solid samples	86
Cu	Al, Cu, Fe, K, Na, Pb	Wine	FAAS	Suitability of ultrafiltration for fractionation study	116
Cu	As, Cu, Fe, Pb	Sugar	FAAS	Decomposition techniques were compared	206
Cu	As, Cu, Mn, Sb, Se	Water	ETAAS	Simultaneous detection and Zeeman background correction	216
Cu	B, Ba, Ce, Cu, Mg, Pb, Sr, Zn	Apple, carrot	ICP-MS, HPLC	SEC-ICP-MS was used for speciation	232
Cu	Cd, Cu	Artificial food digests	ICP-MS	ID, inter-laboratory comparison; RM certification studies	237
Cu	Cd, Cu, Pb	Drinking water	W-coil AAS	Simultaneous determination by AAS	254
Cu	Cd, Cu, Ni, Pb	Drinking water	FAAS	Preconcentration on activated carbon column	256
Cu	Cd, Cu, Pb, Zn	Durum and soft wheat	ETAAS, FAAS	Italian wheat samples	258
Cu	Ca, Cd, Cu, Mg, Pb, Zn	Goat's milk	ETAAS	Samples from Czech Republic	282
Cu	Cd, Cu, Se, Zn	Human milk	AAS	Fortification in fertilizers and effect on maternal milk	284
Cu	Ca, Cd, Cu, K, Mg, Na, P, Zn	Mother milk	FAAS, ETAAS, ICP-OES	*Postpartum* samples; influence of Cd on essential elements	293
Cu	Cd, Cu, Pb	Potable water, plant material	FAAS	Preconcentration by column	304
Cu	Cd, Cu, Pb	Rice	ICP-MS	ID, international measurement evaluation programme	306
Cu	Cd, Cu, Pb, Se	Wheat, wheat products, corn bran, rice flour	ETAAS	MWD procedures were compared	323

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Cu	Co, Cu, Zn	Milk, vitamin samples	FAAS	Batch and column preconcentration	329
Cu	Cu, Fe, Zn	Alcoholic beverage	FAAS	Identification of illegal manufacture	359
Cu	Cu, Fe, Zn	Alcoholic beverages	TXRF	Samples from Venezuela	360
Cu		Animal feed	ETAAS	Direct solid sampling	361
Cu	Cu, Fe, Mn	Beer	FAAS, ICP-OES	Comparison of digestion procedures; MWD	362
Cu		Beer	FAAS	FI; merging zone technique to prevent clogging of burner	363
Cu		Biscuits, bread, cereal	ETAAS	Slurry sampling	364
Cu		Bovine liver	FAAS	Dry aerosol sampling into quartz cell above flame	365
Cu	Cu, Fe	Butter	ETAAS	A method for rapid dissolution and injection	366
Cu		Butter	ETAAS	Butter dispersion was sampled	367
Cu		Butter	ETAAS	Dissolved in butylamine–H_2O–THF	368
Cu	Cu, Fe, Zn	Butter	ETAAS	An anion-exchange column was used to concentrate Cu, Fe, Zn following acid digestion	369
Cu		Carrots	ETAAS	Samples from Japan; geographic origin study	370
Cu	Cu, Zn	Citrus juice	ICP-OES	Elements bound by pectin component	371
Cu	Cu, Fe	Cocoa powder	FAAS	Flow injection	372
Cu	Cu, Zn	Cow and human milk, infant formula	ETAAS	Slurry sampling	373
Cu	Cu, Pb	Dairy products	ETAAS	Slurry sampling	374
Cu		Dairy products	ETAAS	Whole and skimmed milk, and whey	375
Cu	Cu, Mg, Zn	Diabetic diets	AAS	Samples from Japan	376
Cu	Ca, Cu, Fe	Diet	FAAS	Comparison of sample preparation methods	377
Cu	Cu, Fe, Mn, Zn	Diets	AAS	Diets in hospitals; samples from Poland	378
Cu	Cu, Fe, Ni	Edible oils	ICP-OES	Aqueous emulsified sampling	379
Cu	Cu, Ni, Pb	Edible oils, fats	ICP-OES, ETAAS	Atmospheric pressure MWD	380
Cu	Cu, Fe, Zn	Food	FAAS	Dietary intake by toddlers; samples from Belgium	381
Cu		Food, beverages	ICP-MS	Study on deficiency or excess intake, USA	382
Cu	Ca, Cu, Fe, Mg, Zn	Fresh eggs	FAAS	Acid digestion; discrete nebulization	383

Cu	Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Fruits	FAAS	Determinations in 32 fruits	384
Cu	Ca, Cu, K, Mg, Na, P, S	Grape juice, soft drinks	ICP-OES	Discrimination studies	385
Cu		Herbal medicines	ETAAS	Molybdenum tube atomiser, slurry sampling in 10% glycerol	386
Cu	Ca, Cu, Fe, Mg, Zn	Herbs and medicines	FAAS	Chinese samples; therapeutic functions of elements	387
Cu	Cu, Fe, Zn	Human milk	FAAS	*Postpartum* samples; effects of conditions and habits	388
Cu	Cu, Fe, Zn	Human milk	FAAS	Samples from colostrum to the third month of *postpartum*	389
Cu	Cu, Zn	Infant formula, powdered milk, cow milk, human milk	ETAAS	Slurry sampling	390
Cu	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Infant formulas	ICP-OES	For samples and standards, an aqueous medium of 10%(v/v) mixed tertiary amines with EDTA	391
Cu	Ca, Cu, Fe, Mg, Zn	Infant formulas	FAAS	Iron dialysability related to other nutrients	392
Cu	Cu, Fe, Mg	Liver paste	FAAS	MWD, better accuracy than wet ashing	393
Cu		Malt beverages	ETAAS	Rapid method without preconcentration	394
Cu	Cu, Fe, Ni	Margarine	ICP-OES	Emulsion sampling	395
Cu	Ca, Cu, Fe, Mg, P, Zn	Maternal milk	ICP-OES	Emulsified sampling	396
Cu		Milk	ETAAS	Centrifugation then adding $Mg(NO_3)_2$ and Triton X-100	397
Cu	Cu, Mn	Milk	ICP-OES, ETAAS	FI; cationic, anionic and casein-bonded species were determined	398
Cu	Ca, Cu, Fe, Pb	Mouro	FAAS, ETAAS	Characterization and safety study for Greek fruit distillate	399
Cu		Mussels	FAAS	Ultrasound-assisted online FI sampling	400
Cu	Cu, Fe	Oil	FAAS	Flow injection	401
Cu	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Orange juice	ICP-OES	Online focussed MWD using HNO_3; FI	402

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Cu	Cu, Fe	Peanuts	FAAS	Digestion using $H_2SO4–H_2O_2$	403
Cu	Cu, Fe, Zn	Rum	EDXRF	Preconcentration using APDC	404
Cu	Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Skimmed-milk yoghurts	FAAS	Estimation of dietary intakes	405
Cu	Ca, Cu, Fe, K, Mn, Rb, Sr, Sn	Spices	EDXRF	Samples from India	406
Cu	Cu, Fe	Vegetable oils	ETAAS	Extraction using HNO_3 and direct injection	407
Cu		Vegetables	ICP-OES	Pesticide treatment results	408
Cu	Cu, Mn, Pb, Zn	Vegetables, dairy products	FAAS	Online MWD	409
Cu	Cu, Fe, Zn	Vitamin tablets	FAAS	FI for digestion, anion-exchange column preconcentration and separation	410
Cu		Water	FAAS	Online separation	411
Cu		Water, tea	FAAS	Preconcentration by chelation	412
Cu	Cu, Fe, Mn, Zn	Wine	FAAS	Portuguese wines, FI-FAAS	413
Cu	Ca, Cu, Fe, K, Mg, Na	Wine	FAAS	Sweet wines from Canary Islands and Spain were classified	414
Cu		Wine	FAAS, ETAAS, ICP-OES	Comparison of seven different methods for analysis	415
Cu	Cu, Mn, Pb	Wine, drinks	ETAAS	No pretreatment and no chemical modifier	416
F		Diet	ISE	Intake study in Japanese children; duplicate diet technique	417
F		Drinking water		Relation between F content in water and dental health	418
F		Food, beverages, toothpaste		Intake study, 22–25 month-old children	419
F		Food, nutrients		Relations between F intake and fluorosis	420
F		Water		Intake estimation	421
F		Water		Relation between dental disease, F intake and risk of coronary heart disease	422
F		Water	ISE	Trace element content of municipal waters	423

F		Plant foods	ETAAS	Molecular absorption measurement of AlF_3 in graphite furnace	424
Fe	Al, Ca, Cl, K, N, Mg, P, Si	Chicken dung, dates	INAA	Milk SRM supplied by IAEA	89
Fe	Al, Cu, Fe, K, Na, Pb	Wine	FAAS	Suitability of ultrafiltration for fractionation study	116
Fe	As, Cu, Fe, Pb	Sugar	FAAS	Decomposition techniques were compared	206
Fe	Ca, Cd, Ce, Fe, Pb, Zn	Food	ICP-MS	1992 UK survey on diet; quality control in multi-element analysis	272
Fe	Cd, Cr, Fe, Pb	Rice flour	ICP-MS, ID	ID-ICP-MS; cool plasma	310
Fe	Cu, Fe, Zn	Alcoholic beverage	FAAS	Identification of illegal manufacture	359
Fe	Cu, Fe, Zn	Alcoholic beverages	TXRF	Samples from Venezuela	360
Fe	Cu, Fe, Mn	Beer	FAAS, ICP-OES	Comparison of digestion procedures; MWD	362
Fe	Cu, Fe	Butter	ETAAS	A method for rapid dissolution and injection	366
Fe	Cu, Fe, Zn	Butter	FAAS	An anion-exchange column was used to concentrateCu, Fe, Zn following acid digestion	369
Fe	Cu, Fe	Cocoa powder	FAAS	Flow injection	372
Fe	Ca, Cu, Fe	Diet	FAAS	Comparison of sample preparation methods	377
Fe	Cu, Fe, Mn, Zn	Diets	AAS	Diets in hospitals; samples from Poland	378
Fe	Cu, Fe, Ni	Edible oils	ICP-OES	Aqueous emulsified sampling	379
Fe	Cu, Fe, Zn	Food	FAAS	Dietary intake by toddlers; samples from Belgium	381
Fe	Ca, Cu, Fe, Mg, Zn	Fresh eggs	FAAS	Acid digestion; discrete nebulization	383
Fe	Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Fruits	FAAS	Determinations in 32 fruits	384
Fe	Ca, Cu, Fe, Mg, Zn	Herbs and medicines	FAAS	Chinese samples; therapeutic functions of elements	387
Fe	Cu, Fe, Zn	Human milk	FAAS	*Postpartum* samples; effects of conditions and habits	388
Fe	Cu, Fe, Zn	Human milk	FAAS	Samples from colostrum to the third month of *postpartum*	389
Fe	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Infant formulas	ICP-OES	For samples and standards, an aqueous medium of 10%(v/v) mixed tertiary amines with EDTA	391
Fe	Ca, Cu, Fe, Mg, Zn	Infant formulas	FAAS	Iron dialysability related to other nutrients	392
Fe	Cu, Fe, Mg	Liver paste	FAAS	MWD, better accuracy than wet ashing	393
Fe	Cu, Fe, Ni	Margarine	ICP-OES	Emulsion sampling	395

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Fe	Ca, Cu, Fe, Mg, P, Zn	Maternal milk	ICP-OES	Emulsified sampling	396
Fe	Ca, Cu, Fe, Pb	Mouro	FAAS, ETAAS	Characterization and safety study for Greek fruit distillate	399
Fe	Cu, Fe	Oil	FAAS	Flow injection	401
Fe	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Orange juice	ICP-OES	Online focussed MWD using HNO_3; FI	402
Fe	Cu, Fe	Peanuts	FAAS	Digestion using H_2SO_4–H_2O_2	403
Fe	Cu, Fe, Zn	Rum	EDXRF	Preconcentration using APDC	404
Fe	Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Skimmed-milk yoghurts	FAAS	Estimation of dietary intakes	405
Fe	Ca, Cu, Fe, K, Mn, Rb, Sr, Sn	Spices	EDXRF	Samples from India	406
Fe	Cu, Fe	Vegetable oils	ETAAS	Extraction using HNO_3 and direct injection	407
Fe	Cu, Fe, Zn	Vitamin tablets	FAAS	FI for digestion, anion-exchange column preconcentration and separation	410
Fe	Cu, Fe, Mn, Zn	Wine	FAAS	Portuguese wines, FI-FAAS	413
Fe	Ca, Cu, Fe, K, Mg, Na	Wine	FAAS	Sweet wines from Canary Islands and Spain were classified	414
Fe		Beer	FAAS	FI methods were compared	425
Fe	Fe, Zn	Biscuits	FAAS	Evaluation of digestion procedures	426
Fe		Breast milk, infant formula	ETAAS	Speciation by column separation	427
Fe	Fe, Zn	Bulgur wheat	ETAAS	MW heating; $Al_2(SO_4)_3$ as chemical modifier	428
Fe	Fe, Zn	Cereal, legume seeds	FAAS	Effect of soaking on Fe, Zn and phytate content	429
Fe	Ca, Fe	Chinese foods	FAAS	*Postpartum* samples. Effects of high Fe-containing foods	430
Fe		Cooking utensils, apple sauce, hamburger	FAAS	Effect of Fe utensils	431
Fe	Ca, Fe, Mg, Zn	Cow's milk	ICP-OES	Comparison of MWD and treatment with TCA and pepsin	432
Fe	Fe, Zn	Cow's milk	FAAS	High-performance nebulizer, lower LOD	433

Fe	Ca, Fe, Mg	Fast foods	FAAS	Contribution to daily dietary	434
Fe		Flour, milk	FAAS	Intake study, Ivory Coast	435
Fe		Food	AAS	Fe migration from cooking utensils, some was reported	436
Fe	Ca, Fe, K, Mg, Na, Zn	Fruit	FAAS, Flame AES	Lyophilization and slurry sampling	437
Fe		Human milk	ICP-MS	Bioavailability study; fractionation, SEC	438
Fe	Ca, Fe, Zn	Human milk, infant formula	FAAS	Bioavailability study using *in vitro* and *in vivo* methods	439
Fe	Fe, Zn	Human milk, infant formula	FAAS	Bioavailability study	440
Fe	Ca, Fe, Zn	Infant formula, human milk	FAAS	Thickening and bioavailability	441
Fe	Fe, Zn	Legume, grain, vegetable	FAAS	FI, slurry nebulization	442
Fe		Meat	ICP-MS	Speciation, HPLC; haem and non-haem Fe in meat; effect of cooking	443
Fe		Meat	FAAS	Ultrasound-assisted extraction, FI	444
Fe	Ca, Fe	Meat products	AAS	Acceptability of formulated meat product by children; samples from Spain	445
Fe	Ca, Cl, Fe, K, P, S, Zn	Milk and milk-based products	EDXRF	Pellet formation under pressure as sample preparation	446
Fe	Fe, Zn	Rice	ETAAS	Slurry sampling, platform atomization	447
Fe		Rice	ETAAS	Leaves and roots of rice plants; slurry sampling; problems with using TMAH	448
Fe	Fe, Mn	Table olives	FAAS	Validation of a method for industry quality control laboratories	449
Fe	Ca, Fe, Mg, Mn, Zn	Vegetables	FAAS	FI, slurry sampling	450
Fe	Fe, Mn, Zn	Water	FAAS	Column preconcentration	451
Fe		Wine	FAAS	Fe(III)-thiocyanate complex was extracted into MIBK	452
Fe		Wine	FAAS	Cloud point technique for free and tannin-bound species	453
Fe		Wine, grape juice	ETAAS	Method development for routine use. HNO_3–H_2SO_4 digestion was best	454

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Fe		Wine, grape juice and other alcoholic beverages	ETAAS	HNO_3–H_2SO_4 digestion as the best method	455
Hg	As, Cd, Hg	Calcium supplements	CVAAS	Samples from Korea	128
Hg	As, Cd, Hg, Pb	Dietary supplements	ICP-MS	High-resolution ICP-MS	133
Hg	As, Cd, Hg, Pb	Fish	CVAAS	Samples from Adriatic Sea	147
Hg	As, Cd, Hg, Pb	Food	Various	Inter-laboratory study	159
Hg	As, Cd, Hg, Pb	Food	ICP-MS, FAAS	Samples from Catalonia, Spain	160
Hg	As, Cd, Hg, Pb	Medicinal plants	ICP-MS	Samples from Spain	169
Hg	As, Hg, Se, Zn	Seafood	INAA	Samples from Malaysia	189
Hg	As, Hg	Beet sugar	HGAAS, CVAAS	Method development	209
Hg	As, Hg	Sunflower oil	ETAAS	Extracting of species using 0.1 M NH_3–0.01 M EDTA	210
Hg	Cd, Hg	Chinese herbal medicines	HG-AFS	Intermittent flow system; non-dispersive AFS	248
Hg	Cd, Hg, Pb	Diets	CVAAS	Samples from Poland, duplicate diet technique	253
Hg		Baby food, seafood	ETAAS	Slurry sampling	456
Hg		Biological materials	ICP-MS	No sample preparation; ETV for sample introduction; methylmercury and Hg(II)	457
Hg		Biological RMs	ICP-MS	Speciation using CE	458
Hg		Biological RMs	CVAAS	MWD using methanolic KOH; UV oxidation; use of HPLC for methymercury determination	459
Hg		Breast milk	CVAAS	Effects of amalgam filling and dietary intake of fish were examined	460
Hg	Hg, Se	Dogfish CRM	CVAFS, CVAAS	Hg, MeHg and Se were determined	461
Hg		Drinking water	FAAS	Preconcentration on electrode of carbon-coated with gold	462
Hg		Drinking water	CVAAS	Preconcentration, knotted reactor	463
Hg	Se, Hg	Fish	CV-AFS	Bioaccessibility study	464
Hg		Fish	CV-AFS, AFS	Speciation by GC; drying and extraction procedures were compared; problems with using TMAH	465

Hg	Fish	ICP-MS	Protease extraction; speciation by HPLC	466
Hg	Fish	ICP-MS	Speciation by GC-ICP-MS; specific ID calibration; SPME	467
Hg	Fish	MS	Speciation by GC-MS; specific ID calibration; SPME	468
Hg	Fish	ETAAS	Online FI-column preconcentration of methylmercury; complexation with Cu-DDTC	469
Hg	Fish	CVAAS	Organomercury species extracted using toluene	470
Hg	Fish	MIP-AES, GC	Focussed MWD	471
Hg	Fish	Non-instrumental	Coloration of papers for semi-quantitative analysis	472
Hg	Fish	ICP-MS, MIP-AES	Ultrasonication, ethylation and extraction; GC and MIP-AES or ICP-MS	473
Hg	Fish	AFS	Extraction procedures were compared; speciation, capillary GC	474
Hg	Fish	CVAAS	MWD, FI-CVAAS	475
Hg	Fish	GC-AES	Digestion with KOH–methanol	476
Hg	Fish	CVAAS	Inorganic and methylmercury	477
Hg	Fish	CVAAS	Fish from gold mining area, Brazil	478
Hg	Fish	CVAAS	Effects of American-Indian cooking styles	479
Hg	Fish	MIP-AES	MWD; ethylation–extraction into hexane; isothermal separation on a minicolumn	480
Hg	Fish	ETAAS, AAS	FI, SS, Zeeman correction, MWD bomb	481
Hg	Fish	HGAES	Speciation, HG, cryogenic trap-GC-AED method	482
Hg	Fish	AA	Level of Hg in fish species	483
Hg	Fish	CVAAS	Speciation, MeHg, pre-separation by a column	484
Hg	Fish	CVAAS, GC	Samples from Adriatic Sea; total mercury and methylmercury	485
Hg	Fish RMs	ICP-MS	ETV; ultrasonic slurry sampling	486
Hg	Fish, hair	CVAAS	Establishing reference values	487
Hg	Fish, hair	CVAAS, ICP-OES	A multi-vessel system	488
Hg	Fish, urine, blood	ICP-MS	Precipitation of proteins using a virucidal agent; TMAH dissolution	489
Hg	Food colouring agents	ETAAS	Slurry sampling	490
Hg	Food RMs	ETAAS	*In situ* concentration in a Pd–Zr-coated tube	491

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Hg		Food	AES	Methylmercury was determined in food samples; GC-AES	492
Hg		Food	CVAAS	Acid digestion; chemical modification	493
Hg		Food	ETAAS	FI, separation by complex formation	494
Hg		Food	CVAAS	Samples from Poland	495
Hg		Food	Various	Review on trace determination and speciation of Hg	496
Hg		Food	CVAAS	Samples from Japan	497
Hg	Hg, Pb	Human milk	AAS	Samples from Austria	498
Hg		Mushrooms	CVAAS	Wild-edible mushrooms from uncontaminated area, Poland	499
Hg		Mushrooms	CVAAS	Samples from Poland	500
Hg		Mushrooms	CVAAS	Samples from Poland	501
Hg		Mussels	Various	Mussels tissue SRMs	502
Hg		Mussels	Various	RM for Hg and methymercury	503
Hg		Mussels	CVAAS	Speciation; Sephadex G-75 column; Hg(II) and MeHg were determined	504
Hg		Oyster, tuna fish, biological RM, seafood, shellfish	MIP-AES	MW-assisted extraction, cryogenic trapping and GC separation for speciation	505
Hg		Potable water	ICP-MS, CVAFS	Au was used as stabilizing agent to prevent memory effects and to help long-term storage of Hg solutions below 1 ng mL^{-1}	506
Hg		Potable water	ICP-MS	Au was used as stabilizing agent to prevent memory effects and to help long-term storage of Hg solutions	507
Hg		Potable water	ETAAS	Column preconcentration	508
Hg		Seafood	CV-AFS	FI, speciation using a C-18 column and micro-wave conversion to inorganic Hg	509
Hg		Seafood	CVAAS	FI, inter-laboratory study	510

Hg		Vegetable	CVAAS	Rapid digestion by $K_2Cr_2O_7$ in dilute H_2SO_4; better accuracy than AOAC method; full r ecovery of organomercury	511
Hg		Water	ICP-OES	Cloud-point preconcentration, FI-CV-ICP-OES	512
Hg		Water	CV-ICP-MS	Additon of enriched ^{201}-Hg; ^{201}-Hg/^{202}-Hg ratios were measured; bottled drinking water samples from seven countries	513
Hg		Water	CVAAS	Preconcentration on surfactant-coated alumina column and immobilized dithizone	514
Hg		Wild mushrooms	CVAAS	Samples from Poland	515
Hg	Hg, Se	Wine	CVAAS	Samples from Canary Isles	516
Hg		Wine	ETAAS	Liquid extraction; preconcentration	517
Hg		Wines	CVAAS	Amalgamation for preconcentration	518
Hg		Yeast cells	CVAAS	Cells were immobilized onto silica gel	519
I	As, Br, I	Drinking water	ICP-MS	Ion chromatography for speciation	134
I		Cow's milk	ICP-MS	Speciation by IC	520
I		Dairy foods	ICP-MS	Dietary intake study, Germany	521
I		Diet	INAA	Precombustion, iodine is trapped on charcoal	522
I		Food CRMs	ICP-MS	Ashing to convert analyte into non-volatile form	523
I		Food CRMs	ICP-MS, ID	TMAH digestion	524
I		Food	ICP-MS	Extraction by TMAH	525
I		Food	INAA	Methods of extraction for iodine were compared. Iodine was trapped on charcoal	526
I	Br, I	Food, beverages	ICP-MS	Food groups, UK	527
I		Food, SRMs	ICP-MS	Novel spray chamber and nebulizer for lower memory and faster analysis; isotope dilution	528
I		Infant formula, tap water	ETAAS	Ion pair of 1,10-phenanthroline-Hg(II)-I was formed and extracted into IBMK	529
I		Infant formulas	ETAAS	Indirect determination using AgI precipitation	530
I	Br, Cl, I	Milk powder	ICP-OES	MWD, precipitation as silver halides, redissolution in NH_3	531
I		Milk powder	ICP-MS	Solid sampling	532
I		Milk, baby formulase	ETAAS	Iodine was combined with Hg and 2-2′-dipyridyl; complex was extracted into MIBK, Hg was determined; Pd as modifier	533

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
I		Milk, beverages	ICP-MS	Danish diet and beverages; iodine levels in organic and non-organic milk	534
I		Milk, dairy products	ICP-MS	Samples from Norway; seasonal effects	535
I		Milk, infant formula	ICP-MS	Iodine speciation in milk samples from cow, goat, human; SEC	536
I		Milk, infant formula	ICP-MS	Speciation by SEC	537
I		Milk, milk powder	ICP-MS	Rapid and sensitive method	538
I		Nutritional and biological CRMs	ICP-MS	Combustion in a stream of O_2; products were collected in a 5% H_2O–tertiary amine solution	539
I		Oils and fats	INAA	Saturation with bromine followed by INAA of the reacted bromine	540
I		Seafood	ICP-MS	Use of ammonia to stabilize	541
I		Table salt	FAAS	Indirect method by Cr(VI) measurement	542
I		Table salt	EDXRF	Solid sampling	543
I	Cs, I, Sr, Th, U	Total diet	ICP-MS	Inter-laboratory study	544
Mn	Al, Cr, Mn, Mo	Cow's milk, human milk, infant formulas	ETAAS	Slurry sampling	90
Mn	Al, Ba, Mg, Mn	Tea leaf	ICP-OES	Slurry nebulization	114
Mn	As, Cu, Mn, Sb, Se	Water	ETAAS	Simultaneous detection and Zeeman background correction	216
Mn	Cd, Mn, Ni	Mushrooms	ICP-OES	Treated compost, uptake measurements	294
Mn	Cr, Mn	Food	ETAAS	Overnight digestion	341
Mn	Cu, Fe, Mn	Beer	FAAS, ICP-OES	Comparison of digestion procedures; MWD	362
Mn	Cu, Fe, Mn, Zn	Diets	AAS	Diets in hospitals; samples from Poland	378
Mn	Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Fruits	FAAS	Determinations in 32 fruits	384
Mn	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Infant formulas	ICP-OES	For samples and standards, an aqueous medium of 10%(v/v) mixed tertiary amines with EDTA	391
Mn	Cu, Mn	Milk	ICP-OES, ETAAS	FI; cationic, anionic and casein-bonded species were determined	398

Mn	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Orange juice	ICP-OES	Online focussed MWD using HNO_3; FI	402
Mn	Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Skimmed-milk yoghurts	FAAS	Estimation of dietary intakes	405
Mn	Ca, Cu, Fe, K, Mn, Rb, Sr, Sn	Spices	EDXRF	Samples from India	406
Mn	Cu, Mn, Pb, Zn	Vegetables, dairy products	FAAS	Online MWD	409
Mn	Cu, Fe, Mn, Zn	Wine	FAAS	Portuguese wines, FI-FAAS	413
Mn	Cu, Mn, Pb	Wine, drinks	ETAAS	No pretreatment and no chemical modifier	416
Mn	Fe, Mn	Table olives	FAAS	Validation of a method for industry quality control laboratories	449
Mn	Ca, Fe, Mg, Mn, Zn	Vegetables	FAAS	FI, slurry sampling	450
Mn	Fe, Mn, Zn	Water	FAAS	Column preconcentration	451
Mn		Food	FAAS	Study on surfactants and microemulsions	545
Mn		Herbal medicines	ETAAS	Molybdenum atomizer, slurry sampling	546
Mn		Lettuce	ETAAS	MWD, Pd–Md as chemical modifier	547
Mn	Mg, Mn, Zn	Medicinal plants	FAAS, ETAAS	Speciation in aqueous extracts; SEC	548
Mn		Seafood	FAAS	Ultrasound-assisted extraction; FI	549
Mn		Tea leaves, tea	FAAS	Ion-exchange chromatography, FAAS Mn speciation, bioavailability was discussed	550
Mn		Total diet	ETAAS	Dietary intake study, India	551
Mn		Vegetables	FAAS	Preconcentration on activated carbon	552
Mn		Vegetables	FAAS	HNO_3–H_2O_2 digestion or ashed + digested; preconentration, oxine, cupferron, activated carbon	553
Mn		Wine	ETAAS	Contribution to intake in French population	554
Mo	Al, Cr, Mn, Mo	Cow's milk, human milk, infant formulas	ETAAS	Slurry sampling	90
Mo		Edible oils, margarine	INAA	Mo(VI) was extracted from acidic solution using zinc diethyldithiocarbamate in $CHCL_3$	555
Mo		Food, beverages	ICP-OES	Daily dietary intake in Mexico and Germany, duplicate portion sampling	556
Mo		Infant formula	ETAAS, ICP-MS	Mo in infant diets with phenylketonuria	557
Ni	Cd, Ni, Pb, V	Cucumber plants	TXRF	Uptake and transport processes were studied	251

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Ni	Cd, Cu, Ni, Pb	Drinking water	FAAS	Preconcentration on activated carbon column	256
Ni	Cd, Cr, Ni, Pb	Food	ICP-MS	Online dilution for food digests	271
Ni	Cd, Ni, Pb	Food, tissues	ETAAS	MWD	278
Ni	Cd, Ni, Pb	Liver, kidney, food	ETAAS	MWD; chemical modifiers	288
Ni	Cd, Ni, Pb	Mushrooms	ICP-OES	Treated compost, uptake measurements	294
Ni	Co, Cr, Ni	Preserved foods	ETAAS	Platform	330
Ni	Co, Cr, Ni	Vegetables	ETAAS	Sample preparation methods were compared; slurry sampling	331
Ni	Co, Cr, Ni	Wheat flour	ETAAS	Slurry sampling	333
Ni	Cr, Ni	Cooking utensils	ETAAS	Cooking utensils, Cr, Ni leaching from stainless steel pans	335
Ni	Cr, Ni	Food, food utensils	ETAAS	Leaching of Cr and Ni from cooking utensils was studies	340
Ni	Cr, Ni	Wheat flour	ETAAS	Slurry sampling	356
Ni	Cu, Fe, Ni	Edible oils	ICP-OES	Aqueous emulsified sampling	379
Ni	Cu, Ni, Pb	Edible oils, fats	ICP-OES, ETAAS	Atmospheric pressure MWD	380
Ni	Cu, Fe, Ni	Margarine	ICP-OES	Emulsion sampling	395
Ni		Food	FAAS	Online preconcentration using a column	558
Ni		Wines, grapes	ETAAS	Dietary intake measurement	559
Pb	Al, Pb	Fruit juice, milk	FAAS	Preconcentrated on activated carbon-cupferron	100
Pb	Al, Cu, Fe, K, Na, Pb	Wine	Stripping voltammetry	Suitability of ultrafiltration for fractionation study	116
Pb	As, Cd, Hg, Pb	Dietary supplements	ICP-MS	High-resolution ICP-MS	133
Pb	As, Cd, Hg, Pb	Fish	ETAAS	Samples from Adriatic Sea	147
Pb	As, Cd, Pb	Food colours	ETAAS	$NH_4H_2PO_4$ as chemical modifier	155
Pb	As, Cd, Hg, Pb	Food	Various	Inter-laboratory study	159
Pb	As, Cd, Hg, Pb	Food	ICP-MS, FAAS	Samples from Catalonia, Spain	160
Pb	As, Cd, Hg, Pb	Medicinal plants	ICP-MS	Samples from Spain	169
Pb	As, Cd, Pb	Milk powder	AAS, UV-VIS	Secondary reference material	171
Pb	As, Cd, Pb	Seafood	ICP-OES, ICP-MS	A single MWD technique for a variety of seafood samples	188

Pb	As, Cu, Fe, Pb	Sugar	FAAS	Decomposition techniques were compared	206
Pb	As, Pb	Vegetables	ICP-OES, ICP-MS	Samples from Bangladesh; contamination study	214
Pb	As, Cd, Pb	Wine	ICP-MS	Samples from Canary Isles	228
Pb	B, Ba, Ce, Cu, Mg, Pb, Sr, Zn	Apple, carrot	ICP-MS, HPLC	SEC-ICP-MS was used for speciation	232
Pb	Cd, Pb, Se	Baby food	ETAAS	Slurry sampling	238
Pb	Cd, Pb	Beer, carbonated drinks, juice, wine	ETAAS	Samples from Finland	241
Pb	Cd, Pb	Breast milk	ETAAS	MWD	244
Pb	Cd, Pb, Zn	Carrot, endive	FAAS, ETAAS	Samples from Slovenia, effect of power plant	245
Pb	Cd, Pb	Cereals	ICP-MS	Japanese food samples and intake study	246
Pb	Cd, Pb	Cereals, pulses	ICP-MS	North Eastern Chinese products were evaluated	247
Pb	Cd, Pb	Cocoa	ICP-MS	Fractionation using SEC	249
Pb	Cd, Pb	Cocoa	ICP-MS	Bioavailability; *in vitro* gastrointestinal enzymolysis procedure	250
Pb	Cd, Ni, Pb, V	Cucumber plants	TXRF	Uptake and transport processes were studied	251
Pb	Cd, Pb	Diet	ETAAS, ICP-MS	Two techniques are compared; 418 diet homogenate samples	252
Pb	Cd, Hg, Pb	Diets	FAAS	Samples from Poland, duplicate diet technique	253
Pb	Cd, Cu, Pb	Drinking water	W-coil AAS	Simultaneous determination by AAS	254
Pb	Cd, Cu, Ni, Pb	Drinking water	FAAS	Preconcentration on activated carbon column	256
Pb	Cd, Pb	Duplicate diet, food	ETAAS	Samples from Korea	257
Pb	Cd, Cu, Pb, Zn	Durum and soft wheat	ETAAS, FAAS	Italian wheat samples	258
Pb	Cd, Pb	Fish	ETAAS	Samples consumed in Finland	261
Pb	Cd, Pb	Food	ICP-MS	Environmental exposure study, Japan	264
Pb	Cd, Pb	Food CRMs	ETAAS	High-pressure ashing and MWD were compared	267
Pb	Cd, Cr, Pb	Food packaging	ICP-MS	Paper and paper board analysis; MWD	268
Pb	Cd, Pb	Food SRMs	ICP-MS, HGAAS, CVAAS	High-pressure ashing and MWD were compared	269
Pb	Cd, Pb	Food	ETAAS	Cryogenic grinding and slurry sampling, Rh–W-coated graphite tubes	270
Pb	Cd, Cr, Ni, Pb	Food	ICP-MS	Online dilution for food digests	271
Pb	Ca, Cd, Ce, Fe, Pb, Zn	Food	ICP-MS	1993 UK survey on diet; quality control in multi-element analysis	272

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Pb	Cd, Pb	Food	FAAS	Beam injection flame furnace AAS, slurry sampling	273
Pb	Cd, Ni, Pb	Food, tissues	ETAAS	MWD	278
Pb	Cd, Pb	Foodstuffs	ETAAS	Multi-element AAS	279
Pb	Cd, Pb	Foodstuffs	ETAAS	Duplicate diet study; samples from Korea	280
Pb	Cd, Pb	Fruit juice	ETAAS	Home-made ETAA spectrometer	281
Pb	Ca, Cd, Cu, Mg, Pb, Zn	Goat's milk	ETAAS	Samples from Czech Republic	282
Pb	Cd, Pb	Infant cereals	ETAAS	Dry ashing and acid dissolution before injection	285
Pb	Cd, Ni, Pb	Liver, kidney, food	ETAAS	MWD; chemical modifiers	288
Pb	Cd, Pb	Lupin seeds	ETAAS	Australian lupin seeds	289
Pb	Cd, Pb	Medicinal plants	AAS	Samples from India	291
Pb	Cd, Cr, Pb, Zn	Milk powder	ICP-MS	Ultrasonic slurry sampling; ETV and reaction cell	292
Pb	Cd, Pb	Mussels	ETAAS	Extraction from tissue using dilute HNO_3 and ultrasound	296
Pb	Cd, Pb	Mustard plants	ICP-OES	Uptake from soil	299
Pb	Cd, Cu, Pb	Potable water, plant material	FAAS	Preconcentration by column	304
Pb	Cd, Cu, Pb	Rice	ICP-MS	ID, international measurement evaluation programme	306
Pb	Cd, Cr, Fe, Pb	Rice flour	ICP-MS, ID	ID-ICP-MS; cool plasma	310
Pb	Cd, Pb	Sunflower oil	ETAAS	Digestion using HNO_3–H_2O_2; 21 samples	313
Pb	Cd, Pb, Zn	Vegetable oil	ICP-MS	ETV-ICP-MS; emulsion sampling	316
Pb	Cd, Pb	vegetables, protein foodstuff	ETAAS	MWD, Pd as MM	318
Pb	Cd, Pb	Water	ETAAS	Batch preconcentration, slurry sampling	319
Pb	13-C, Cd, 15-N, Pb, Se, Sr	Wheat	ICP-MS	Identification of geographic origin	321
Pb	Cd, Cu, Pb, Se	Wheat, wheat products, corn bran, rice flour	ETAAS	MWD procedures were compared	323

Pb	Cd, Pb	Wine	ETAAS	Transverse heating and Zeeman effect background correction; Pd and Mg as chemical modifiers	324
Pb	Cd, Pb	Wines	ETAAS	MWD in concentrated nitric acid; samples from Korea	326
Pb	Cr, Pb	Drinking water	ETAAS	Preconcentration in electrokinetic flow system	336
Pb	Cu, Pb	Dairy products	ETAAS	Slurry sampling	374
Pb	Cu, Ni, Pb	Edible oils, fats	ICP-OES, ETAAS	Atmospheric pressure MWD	380
Pb	Ca, Cu, Fe, Pb	Mouro	FAAS, ETAAS	Characterization and safety study for Greek fruit distillate	399
Pb	Cu, Mn, Pb, Zn	Vegetables, dairy products	FAAS	Online MWD	409
Pb	Cu, Mn, Pb	Wine, drinks	ETAAS	No pretreatment and no chemical modifier	416
Pb	Hg, Pb	Human milk	AAS	Samples from Austria	498
Pb		Baby food	ETAAS	Digestion procedures were compared	560
Pb		Beverages	FAAS	Column preconcentration; online copper removal	561
Pb		Biological RMs	ICP-MS	Pb isotopes were determined by magnetic sector MS. ^{209}-Bi was internal standard	562
Pb		Ca supplements	ETAAS, ICP-MS	Ca interference was eliminated using a sector field ICP-MS	563
Pb		Canned tuna	FAAS	Evaluation of AOAC method	564
Pb		Dietary Ca supplements	ETAAS	MWD, 55 brands	565
Pb		Drinking water	ICP-OES	USN; preconcentration on resin; FI	566
Pb		Drinking water	ETAAS	Samples from Poland; contamination from lead piping	567
Pb		Edible wild mushrooms	ETAAS	Samples from Spain; contamination due to traffic pollution	568
Pb	Pb, Zn	Food	ICP-MS	FI, simple method, bioaccessibility	569
Pb		Food	HGAAS	Comparison with FAAS	570
Pb		Food, beverages, milk	HG-ICP-OES	Effects of acetic, citric, nitric and tartaric acids on hydride formation	571
Pb		Herbs	FAAS	Trapping on cooled silica tube; Chinese herbs	572
Pb		Honey	ICP-OES	Preconcentration on a minicolumn	573
Pb		Honey	ETAAS	Comparison of chemical modifiers	574
Pb		Human milk	ETAAS	Samples from Egypt; WHO levels exceeded; effect of Pb plumbing	575

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Pb		Infant formula, infant food	ETAAS	Old plumbing, contamination of tap water used in samples	576
Pb		Medicinal plants	ETAAS	Samples from Malaysia	577
Pb		Milk	ETAAS	Dilution with Triton X-100; Pd and $Mg(NO_3)_2$ as modifiers	578
Pb		Port wine	ICP-MS	Pre-treatment by UV irradiation; isotope ratios were used for dating	579
Pb		Powder drinks, honey, syrups	ETAAS	End-capped THGA-AAS	580
Pb		Raisins	ETAAS	Contaminated copper fungicide caused high levels	581
Pb		Rice	ETAAS	World samples, seventeen areas, 1990–1995, last 10 years no reduction in Pb levels	582
Pb		Seafood	FAAS	Minicolumn online preconcentration	583
Pb		Tap water	FAAS	Comparison of sorbents for preconcentration	584
Pb		Tap water	FAAS	Preconcentration on knotted reactor; FI	585
Pb		Tap water	ICP-OES, FI	Preconcentration as Pb-DDC complex	586
Pb		Turkish cookies	ETAAS	(W+Pd+tartaric acid) as matrix modifier	587
Pb		Vinegar	ICP-MS-ETAAS	Different sample preparation procedures	588
Pb	Pb, Sb, Tl	Water	ICP-OES	Chelation, extraction	589
Pb		Water	ETAAS	Chemical modifier	590
Pb		Water	FAAS	Online preconcentration, slotted quartz tube	591
Pb		Water	ICP-OES	Molecular imprinting; preconcentration on resins	592
Pb		Water	FAAS	FI; online ion exchange and extraction for preconcentration	593
Pb		Water	ETAAS	Effect of coatings on graphite tube; Pd as chemical modifier	594
Pb		Water, tea CRM, herbal medicines	FAAS	Preconcentration using APDC in a knotted reactor	595
Pb		Wine	ICP-MS	Isotope ratios were determined; use of UV irradiation for sample pretreatment	596
Pb		Wine	ICP-MS	Isotope ratios were determined using TOF-ICP-MS	597

Pb		Wine	ICP-OES	Column preconcentration, USN	598
Pb		Wine	PIXE	Liquid samples on filter paper were sampled	599
Pb		Wine	ICP-MS	Inter-laboratory study	600
Pb		Wine	ICP-MS	ID, agreed with TIMS	601
Pb		Wine	ETAAS	Samples from Australia; systematic assay of grapes, must and wine	602
Pb		Wine	ICP-MS	Isotope ratios in classification; samples from Italy; geographic origin study	603
Pb		Wine	ICP-MS	Isotopic ratios for region of origin	604
Pb		Wine	HGAAS	Online MW digestion; FI; $HNO_3 + H_2O_2$ digestion; Spanish wines	605
Pb		Wine	ETAAS	W(VI), Pd(II), HNO_3 as CM	606
Pb		Wine	ICP-MS	Speciation, SEC	607
Pb		Wine	ETAAS	Pb migration from wine glasses	608
Pb		Wine	ETAAS	Pd modifier; causes of Pb contamination	609
Pb		Wine	FAAS	FI, online column separation; speciation	610
Pb		Wine	ETAAS	Comparison of chemical modifiers	611
Pb		Wine	ETAAS, ICP-MS	Inter-laboratory study	612
Pb		Wines	ICP-MS, ETAAS	Samples from Portugal	613
Pb		Wines	ICP-MS	Isotope ratios; TOF, Sector Field and Q-ICP-MS were compared	614
Se	As, Se	Coffee	HG-ICP-OES	MWD using HNO_3 and H_2O_2	131
Se	As, Se	Egg powder, mussel tissue, nuts	ICP-MS, ETAAS, ICP-OES, HGAAS	Comparison of analytical techniques and digestion procedures	145
Se	As, Se	Fish	ICP-MS, HPLC	Speciation using HPLC-ICP-MS	146
Se	As, Se, Sb, Te	Fish, water SRMs	ICP-MS	Ion-exchange HPLC for speciation	152
Se	As, Se	Food	HGAAS	CF, FI, cryogenic trapping	153
Se	As, Se	Food	HGAAS	MWD, dry ashing	158
Se	As, Bi, Sb, Se, Te	Milk	HG-AFS	Slurry sampling	170
Se	As, Se	Milk SRM	ICP-MS	Tertiary amine digestion; polyatomic interference study	173
Se	As, Hg, Se, Zn	Seafood	INAA	Samples from Malaysia	189
Se	As, Cu, Mn, Sb, Se	Water	ETAAS	Simultaneous detection and Zeeman background correction	216

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Se	As, Se	Water	ICP-MS	Speciation, HPLC	217
Se	As, Se	Water	HG-ICP-MS	Speciation, CE; online reduction	218
Se	As, Se	Water, urine, kelp RMs	HG-ICP-MS	Pneumatic nebulization and hydride introduction; speciation	225
Se	Cd, Pb, Se	Baby food	ETAAS	Slurry sampling	238
Se	Cd, Cu, Se, Zn	Human milk	AAS	Fortification in fertilizers and effect on maternal milk.	284
Se	13-C, Cd, 15-N, Pb, Se, Sr	Wheat	ICP-MS	Identification of geographic origin	321
Se	Cd, Cu, Pb, Se	Wheat, wheat products, corn bran, rice flour	ETAAS	MWD procedures were compared	323
Se	Hg, Se	Dogfish CRM	CVAFS, CVAAS	Hg, MeHg and Se were determined	461
Se	Se, Hg	Fish	ICP-MS, HG-AFS	Speciation, HPLC; bioaccesibility study	464
Se	Hg, Se	Wine	HGAAS	Samples from Canary Isles	516
Se	Sb, Se	Baker's yeast	ICP-MS, HGAAS	Bioaccumulation study; effect of oxidation state	615
Se		Brassica	ICP-MS, ES-MS	Study on phytoremediation, HPLC was used	616
Se		Brazil nuts	ICP-MS, ES-MS	Speciation, LC	617
Se		Broccoli	ICP-MS	Extraction methods were compared; speciation by HPLC	618
Se		Cereals	ICP-MS	Fertilizers and products, Austrian study	619
Se		Coconut milk, coconut water	ETAAS	Direct extraction of Se from samples suing a mixture of tertiary amines, Pd modifier	620
Se		Cod	ICP-MS	Speciation, HPLC; ID, comparison of extraction procedures	621
Se		Cod, gastric fluid	ICP-MS	Speciation, HPLC; gastric enzymolysis to digest	622
Se		Coffee, coffee beans	ICP-MS	Speciation by SPME and GC	623
Se		Cow's milk, infant formula	HGAAS	Samples were taken over a period of 11 years, Japanese study	624
Se		Dairy products, fruits, vegetables	HGAAS	MWD, FI	625
Se		Dietary Se supplement	ICP-MS	HPLC-ICP-MS; chiral speciation	626

Se	Dietary supplements	ETAAS, HGAAS	Comparison of techniques; Cu, Mn and Zn nterferences in HGAAS	627
Se	Dietary supplements	ICP-MS	Speciation, CE, HPLC	628
Se	Dietetic compounds	ICP-OES	Formation of volatile brominated compounds using Br^- in H_2SO_4	629
Se	Drinking water	GC-MS	Derivatization and SPME	630
Se	Fish	ICP-MS	Speciation; SEC-ICP-MS	631
Se	Fish	ETAAS	Slurry sampling, MM ammonium oxalate, freeze drying	632
Se	Fish	HGAAS	Samples from Adriatic Sea	633
Se	Fish, shellfish, clover, flour, yeast	ICP-MS	Speciation, ion chromatography, SEC	634
Se	Food, beverage	HGAAS	Samples from Southern Spain	635
Se	Food, diet	HG-AFS	Samples from Slovenia	636
Se	Food	HG-AFS	Conversion to Se(IV) by MWD	637
Se	Food	HGAAS	Dietary intake study, Austria and Slovenia	638
Se	Food	HGAAS	Dietary intake in Ireland and other countries	639
Se	Food	ETAAS	Closed vessel acid digestion; column separation by complex formation	640
Se	Food	ETAAS	MM, Pt(IV) and $MgNO_3$	641
Se	Food	HGAAS	Samples from Croatia	642
Se	Food	W-coil AAS	Preconcentration on cobalt oxide	643
Se	Fruit juice	ETAAS	$HCl–HNO_3$, 3+1 digestion, Ni–Pt as best MM	644
Se	Fruit juice	HGAAS	Online MW reduction of Se(VI) to Se(IV); speciation	645
Se	Garlic	Flame AFS, HPLC	Speciation by HPLC, C-18 column	646
Se	Garlic	ICP-MS	Speciation, multi-mode gel filtration HPLC columns	647
Se	Garlic, yeast	ICP-MS, HPLC, HPLC-ES-MS	Speciation using MS techniques	648
Se	Garlic, yeast	ICP-MS	Speciation, HPLC-ES-ICP-MS	649
Se	Green onion	ICP-MS	Enriched samples; speciation, ion-pair HPLC, SEC	650
Se	Human milk	AAS??	Se in Kuwaiti mothers' milk	651

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Se		Human milk	HGAAS	Milk, blood, MWD, FI-HGAAS	652
Se		Human milk	HGAAS	20 volunteers, intake of breast-fed infants	653
Se		Infant formula	ETAAS	Digestion, ashing, pre-reduction	654
Se		Infant formula	ETAAS, HGAAS	Adequate intake study	655
Se		Meat	HGAAS	Acid digestion overnight	656
Se	Se, Te	Milk	HG-AFS	MWD	657
Se		Milk, infant formulas	Various	Review for Se determination; effects of reduction of Se(VI) to Se(IV)	658
Se		Mineral water	HGAAS, HG-AFS	Preconcentration on a gold wire	659
Se		Mushrooms	AFS	Comparison of extraction procedures; speciation using HPLC	660
Se	Cs, Se	Mushrooms	XRF	Minimum sample preparation	661
Se		Mussel, wheat flour SRM	ETAAS, HGAAS	TMAH digestion, speciation	662
Se		Nutrition liquids	ETAAS	HG-ETAAS, Se in selenosugar. Hydrides were trapped in a Pd-coated graphite cuvette	663
Se		Nutritional supplements	ICP-MS	Speciation, GC; esterification and acylation	664
Se		Nuts	ICP-MS	Speciation using HPLC in pecan, cashew and walnut from Brazil	665
Se		Onion leaves	ICP-MS	Speciation, HPLC	666
Se		Onions, potatoes	PIXE	Dissolution and deposition on polycarbonate	667
Se		Oysters	ICP-MS	Comparison of extraction procedures; speciation using HPLC, use of mass cut-off filters	668
Se		Oysters	ICP-MS, ETAAS	Speciation, HPLC	669
Se		Plant tissues	HG-ICP-MS	MWD digestion using HNO_3–HF–H_2O_2	670
Se		Plants	MIP-AES	Speciation, multicapillary GC	671
Se		Rice	HG-AFS	Fortification study using foliar application	672
Se		Rice	HG-AFS	Fortification study using foliar application	673
Se		Seafood	HG-AFS	Enzyme extraction, MWD, speciation using HPLC	674
Se		Seafood	ETAAS	Ultrasonic extraction, no pyrolysis stage, Pd modifier	675

Se	Seafood	ICP-OES	Human blood and seafood Se levels were related; samples from Taiwan	676
Se	Se-enriched onion, garlic and yeast	ICP-MS	HPLC-ICP-MS; chiral speciation	677
Se	Serum, breast milk	ICP-MS	Speciation; capillary zone electrophoresis for separation of species	678
Se	Se-yeast	ICP-MS	Speciation, HPLC; stability and reactivity of Se species	679
Se	Se-yeast	ICP-MS	Speciation, CE	680
Se	Se-yeast, Se-enriched supplements	ICP-MS, MS-MS	Speciation, anion-exchange and ion-pair HPLC	681
Se	Soya milk	HGAAS	Digestion, HCl + HNO_3	682
Se	Soyabean, okra	HG-AFS	Soil supplementation, foliar application and Se contents in samples	683
Se	Tissue, food, fish	ETAAS	Enzyme extraction, MM Pd–citric acid	684
Se	Vegetables	ICP-MS	Speciation, HPLC; Se-enriched vegetables	685
Se	Vegetables	HG-AFS	Samples from Slovenia	686
Se	Water	HGAAS, HG-ICP-OES, HG-ICP-MS	Speciation, HPLC; focussed MWD	687
Se	Wheat	MIP-AES	Selenomethionine; protein hydrolysis; TMAH, Al_2O_3; MIP-AES, FPD, MS compared	688
Se	Wheat, rye, cocoa butter	INAA	Radiochemical separation at low levels	689
Se	White clover	ICP-OES, ETAAS	Speciation, microbore ion-exchange chromatography; samples grown on Se-enriched soil	690
Se	Wine	ETAAS	Pd, hydroxylammonium chloride as modifiers	691
Se	Wine	ETAAS	Ni and Sr as chemical modifiers	692
Se	Yeast	ICP-MS	HPLC-ICP-MS; chiral speciation	693
Se	Yeast	HPLC-ES-MS, HPLC-ICP-MS	Speciation using MS techniques	694
Se	Yeast	ICP-MS, HPLC	Extraction procedures were evaluated; a sequential procedure was needed for a good recovery	695
Se	Yeast	ICP-MS	HPLC-ICP-MS, enzyme extraction	696

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Se		Yeast	ICP-MS, ES-LC-MS	Extraction with cold $HClO_4$; speciation, HPLC	697
Se		Yeast	FAAS, HGAAS, ICP-MS, INAA	Comparison of measurement and digestion procedures; use of $HClO_4$ and adverse effect on recovery	698
Se		Yeast	ICP-MS, LC-MS	Speciation, SEC, HPLC, nanospray	699
Se		Yeast	ICP-MS	Speciation by HPLC and gel electrophoresis; ETV for sample introduction; Se-enriched yeast candidate RM	700
Se		Yeast	ICP-MS	Speciation, SEC, HPLC	701
Se		Yeast	HG-AFS	Speciation, HPLC; MWD	702
Se		Yeast	ETAAS	MM, palladium nitrate and magnesium nitrate	703
Se		Yeast	ICP-MS	Speciation, HPLC; electrospray MS	704
Se		Yeast	MALDI, MS	Speciation, HPLC; systematic approach	705
Se		Yeast	HG-AFS, ICP-MS	Speciation, ion-pair HPLC, MW-assisted system	706
Se		Yeast	ICP-MS	Speciation, CNBr derivatization, ID, GC-ICP-MS, GC-MS	707
Se		Yeast	ICP-MS, MIP-AES, AFS	SPME multicapillary GC with different detectors; speciation	708
Se		Yeast	ICP-MS	Use of ETV, speciation by SDS–PAGE	709
Se		Yeast extracts	ICP-MS, ES-MS	Sequential use of SEC, anion-exchange and cation-exchange for speciation	710
Se		Yeast-based food supplements	ICP-MS	Speciation, HPLC	711
Si	Al, Ca, Cl, Fe, K, N, Mg, P	Chicken dung, dates	INAA	Milk SRM supplied by IAEA	89
Si	Al, Ba, Si, Sr, Ti	Food	ICP-OES	Direct fusion using lithium metaborate	94
Si	Na, Sn, Si	Citrus juice	ICP-OES	Comparison of extraction techniques	712
Si		Food	Several	Inter-laboratory study	713
Si		Food	ETAAS	Bioavailability study	714
Si		Foodstuffs	AAS	Duplicate diet method, Belgian adults	715

Si		Human milk	ETAAS	Patients with silicone implants and controls	716
Si		Milk, infant formula	ETAAS	Pd as chemical modifier	717
Si		Tap water	ETAAS	Chemical modifier	718
Sn	Ca, Cu, Fe, K, Mn, Rb, Sr, Sn	Spices	EDXRF	Samples from India	406
Sn	Na, Sn, Si	Citrus juice	ICP-OES	Comparison of extraction techniques	712
Sn		Canned foods	ETAAS	Electrodeposition on a tungsten probe; probe atomization	719
Sn		Canned foods	ICP-OES	High-pressure ashing and MWD; novel methods	720
Sn		Diet, oyster	ETAAS	Ultrasonic probe extraction	721
Sn		Food	GC-AES	Butyltin compunds were determined; samples from China	722
Sn		Fruit, vegetable, juices	ICP-MS	Effects of packaging	723
Sn		Fruits	ICP-MS	Sn as an indicator for presence of pesticides	724
Sn		Mussels	ETAAS	Lyophilization and digestion by HNO_3, HF and $HClO_4$ for digestion, Ni as stabilizer	725
Sn		Mussels, sea urchin eggs	GC-AES, GC-FPD	Speciation; TBT and trimethyltin using GC-AES and GC-FPD, respectively	726
Sn		Oyster tissue	ICP-MS	Speciation by HPLC-ID-ICP-MS; TBT was determined	727
V	Cd, Ni, Pb, V	Cucumber plants	TXRF	Uptake and transport processes were studied	251
V		Cow's milk, human milk, infant formula	ETAAS		728
V		Food, Chinese herbal remedies	ETAAS	Method optimization	729
V		Wine	ETAAS	Dietary intake measurement	730
Zn	Al, Zn	Infant formula, infant food	FAAS	MWD	103
Zn	As, Hg, Se, Zn	Seafood	INAA	Samples from Malaysia	189
Zn	B, Ba, Ce, Cu, Mg, Pb, Sr, Zn	Apple, carrot	ICP-MS, HPLC	SEC-ICP-MS was used for speciation	232
Zn	Cd, Zn	Bovine offal, muscle	ETAAS	Low levels in samples from organically reared	242
Zn	Cd, Pb, Zn	Carrot, endive	FAAS, ETAAS	Samples from Slovenia, effect of power plant	245
Zn	Cd, Cu, Pb, Zn	Durum and soft wheat	ETAAS, FAAS	Italian wheat samples	258
Zn	Ca, Cd, Ce, Fe, Pb, Zn	Food	ICP-MS	1994 UK survey on diet; quality control in multi-element analysis	272

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Zn	Ca, Cd, Cu, Mg, Pb, Zn	Goat's milk	FAAS	Samples from Czech Republic	282
Zn	Cd, Cu, Se, Zn	Human milk	AAS	Fortification in fertilizers and effect on maternal milk	284
Zn	Cd, Cr, Pb, Zn	Milk powder	ICP-MS	Ultrasonic slurry sampling; ETV and reaction cell	292
Zn	Ca, Cd, Cu, K, Mg, Na, P, Zn	Mother milk	FAAS, ETAAS, ICP-OES	*Postpartum* samples; influence of Cd on essential elements	293
Zn	Cd, Pb, Zn	Vegetable oil	ICP-MS	ETV-ICP-MS; emulsion sampling	316
Zn	Co, Cu, Zn	Milk, vitamin samples	FAAS	Batch and column preconcentration	329
Zn	Cu, Fe, Zn	Alcoholic beverage	FAAS	Identification of illegal manufacture	359
Zn	Cu, Fe, Zn	Alcoholic beverages	TXRF	Samples from Venezuela	360
Zn	Cu, Fe, Zn	Butter	FAAS	An anion-exchange column was used to concentrate Cu, Fe, Zn following acid digestion	369
Zn	Cu, Zn	Citrus juice	ICP-OES	Elements bound by pectin component	371
Zn	Cu, Zn	Cow and human milk, infant formula	ETAAS	Slurry sampling	373
Zn	Cu, Mg, Zn	Diabetic diets	AAS	Samples from Japan	376
Zn	Cu, Fe, Mn, Zn	Diets	AAS	Diets in hospitals; samples from Poland	378
Zn	Cu, Fe, Zn	Food	FAAS	Dietary intake by toddlers; samples from Belgium	381
Zn	Ca, Cu, Fe, Mg, Zn	Fresh eggs	FAAS	Acid digestion; discrete nebulization	383
Zn	Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Fruits	FAAS	Determinations in 32 fruits	384
Zn	Ca, Cu, Fe, Mg, Zn	Herbs and medicines	FAAS	Chinese samples; therapeutic functions of elements	387
Zn	Cu, Fe, Zn	Human milk	FAAS	*Postpartum* samples; effects of conditions and habits	388
Zn	Cu, Fe, Zn	Human milk	FAAS	Samples from colostrum to the third month of *postpartum*	389
Zn	Cu, Zn	Infant formula, powdered milk, cow milk, human milk	ETAAS	Slurry sampling	390

Zn	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Infant formulas	ICP-OES	For samples and standards, an aqueous medium of 10% (v/v) mixed tertiary amines with EDTA	391
Zn	Ca, Cu, Fe, Mg, Zn	Infant formulas	FAAS	Iron dialysability related to other nutrients	392
Zn	Ca, Cu, Fe, Mg, P, Zn	Maternal milk	ICP-OES	Emulsified sampling	396
Zn	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Orange juice	ICP-OES	Online focussed MWD using HNO_3; FI	402
Zn	Cu, Fe, Zn	Rum	EDXRF	Preconcentration using APDC	404
Zn	Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Skimmed-milk yoghurts	FAAS	Estimation of dietary intakes	405
Zn	Cu, Mn, Pb, Zn	Vegetables, dairy products	FAAS	Online MWD	409
Zn	Cu, Fe, Zn	Vitamin tablets	FAAS	FI for digestion, anion-exchange column preconcentration and separation	410
Zn	Cu, Fe, Mn, Zn	Wine	FAAS	Portugese wines, FI-FAAS	413
Zn	Fe, Zn	Biscuits	FAAS	Evaluation of digestion procedures	426
Zn	Fe, Zn	Bulgur wheat	ETAAS	MW heating; $Al_2(SO_4)_3$ as chemical modifier	428
Zn	Fe, Zn	Cereal, legume seeds	FAAS	Effect of soaking on Fe, Zn and phytate content	429
Zn	Ca, Fe, Mg, Zn	Cow's milk	ICP-OES	Comparison of MWD and treatment with TCA and pepsin	432
Zn	Fe, Zn	Cow's milk	FAAS	High-performance nebulizer, lower LOD	433
Zn	Ca, Fe, K, Mg, Na, Zn	Fruit	FAAS, Flame AES	Lyophilization and slurry sampling	437
Zn	Ca, Fe, Zn	Human milk, infant formula	FAAS	Bioavailability study using *in vitro* and *in vivo* methods	439
Zn	Fe, Zn	Human milk, infant formula	FAAS	Bioavailability study	440
Zn	Ca, Fe, Zn	Infant formula, human milk	FAAS	Thickening and bioavailability	441
Zn	Fe, Zn	Legume, grain, vegetable	FAAS	FI, slurry nebulization	442
Zn	Ca, Cl, Fe, K, P, S, Zn	Milk and milk-based products	EDXRF	Pellet formation under pressure as sample preparation	446
Zn	Fe, Zn	Rice	ETAAS	Slurry sampling, platform atomization	447
Zn	Ca, Fe, Mg, Mn, Zn	Vegetables	FAAS	FI, slurry sampling	450
Zn	Fe, Mn, Zn	Water	FAAS	Column preconcentration	451

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Zn	Mg, Mn, Zn	Medicinal plants	FAAS, ETAAS	Speciation in aqueous extracts; SEC	548
Zn	Pb, Zn	Food	ICP-MS	FI, simple method, bioaccessibility	569
Zn		Food	ETAAS, FAAS	Acid digestion or two-stage enzymatic digestion	731
Zn		Food	AAS	Studies regarding healthy children from Poland	732
Zn		Food	HG-AFS	Hydride generation from organized medium of cetyltrimethylammonium bromide	733
Zn		Food	FAAS	Dietary intake, Egypt; phytate was also determined to asses its impact on Zn bioavailability	734
Zn	Ca, Mg	Honey	FAAS, FI	Dissolution in dilute HCl containing La; FI	735
Zn		Human milk	ETAAS	Dilution with 0.1% Triton X-100	736
Zn		Infant food	FAAS	Zn bioavailability study. Phytate has a negative effect	737
Zn		Milk	ICP-OES	Comparison of sample decomposition procedures	738
Zn		Milk fat	FAAS	Emulsification for sampling	739
Zn	Ca, Mg, P, Zn	Yoghurt	FAAS	Elemental distribution between soluble and micellar fractions	740
Various	Cd, Cr, Cu, Ni, Pb, Zn	Aceto balzamico	FAAS	Metals were extracted by a butylamine/water solution	741
Various	Ag, As, Co, Cu, Mn, Ni, Se, Te, V	Animal tissues	ICP-MS	ETV; slurry atomization; dissolution using TMAH	742
Various	B, Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Avocado	FAAS, ETAAS	Area of origin by element concentrations	743
Various	As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, Zn	Beef, pork	HGAAS	Food imported into Sweden from six countries	744
Various	Ag, Cd, Cu, Fe, In, Mn, Pb, Tl, Zn	Beer	FAAS	Atom trapping on water-cooled silica tube	745
Various	Cd, Co, Cu, Ni, Zn	Beer	ICP-MS	Offline ion exchange, preconcentration	746
Various	As, Sb, Se, Sn, Hg	Beer wort	HGAAS, CVAAS, ETAAS	Vapour generation and trapping in Au- or Pd-treated graphite furnace	747

Various	As, Cd, Co, Cr, Cu, Pb, Sn, Zn	Beet sugar	ETAAS	Carbon was removed using oxygen at pyrolysis step, Pd and ascorbic acid as chemical modifiers	748
Various	Co, Cr, Cu, Fe, Pb, Zn	Brazilian beers	ICP-OES	Digestion methods were compared	749
Various	Cd, Cu, Fe, Pb, Zn	Bread	FAAS, ETAAS	Samples from Turkish bakeries	750
Various	Al, Br, Ca, Cl, Fe, Mg, Mn, Na, Rb, Sb, Zn	Bread, milk powder	INAA	Samples from Brazil	751
Various	Ca, Cu, Fe, Mg, Mn, Zn	Breast milk	ICP-OES	Speciation; separation of complexes using SEC; ultrasonic nebulization; 60 Italian mothers	752
Various	Al, Cd, Co, Cu, Mn, Ni, Se, Zn	Breast milk	ICP-MS, ETAAS	Elimination of matrix problems using a double focussing instrument	753
Various	Cu, Fe, Mn, Se, Zn	Camel, cow, human milk, infant formula	FAAS, ETAAS	Milk samples from Kuwait	754
Various	Al, Cd, Cr, Cu, Mn, Ni, Pb, V, Zn	Composite diet samples	ICP-MS	Measurements with low-resolution ICP-MS	755
Various	Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn	Dates	ICP-MS	Samples from Saudi Arabia	756
Various	Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, P, Zn	Diets	ICP-OES	Nigerian duplicate diet study	757
Various	Cd, Cu, Ni, Pb	Drinking water	XRF	Preconcentration by complex formation	758
Various	Al, Cd, Cr, Cu, Pb, Zn	Drinking water	AAS	Samples from Brazil	759
Various	As, Cd, Ni, Pb	Durum wheat, semolina, pasta	ICP-MS	Levels in commercial samples; effects of milling, processing and cooking	760
Various	Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Edible seaweeds	FAAS	Dry ashing	761
Various	Al, Cd, Cr, Cu, K, Mg, Mn, Pb, Rb, Sr	Flour	AES	Laser induced breakdown spectrometry, direct determination using solid samples	762
Various		Food	TXRF	Review for multi-element analysis	763
Various		Food	Various	Review on speciation in food samples	764
Various		Food RMs	Various	Review for certification; speciation	765

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Various	Cd, Cu, Mn, Pb, Zn	Food SRMs	FAAS	Online digestion using heated Pt–Ir capillary; use of HF; FI	766
Various	Cd, Co, Cu, Mn, Ni, Pb, Zn	Food, vegetables	FAAS	Relation between soil and product content; possibility of effects on gastrointestinal cancers; samples from Van region, Turkey	767
Various	Al, As, Cd, Hg, Pb, Se	Food	ICP-MS	High-pressure ashing; 2% isopopyl alcohol to overcome interferences from residual C	768
Various		Food	Various	Food authenticity studies; review	769
Various		Food	MS	Review; several MS techniques in food analysis	770
Various		Food	PIXE, EDXRF, AAS	Preparation and certification of CRMs	771
Various	Cd, Cr, Cu, Fe, Ni, Pb, Zn	Food	AAS	Inter-laboratory study, 16 labs, five kinds of food, two kinds of composite diet	772
Various	As, Be, Cd, Ce, Cr, Hg, Pb, Sb, Sn	Food	ICP-MS	Hospital diets were examined	773
Various	Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn	Food	FAAS, ETAAS	Organic matrix was destroyed by a wet procedure	774
Various	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Food	ICP-OES	Four food groups used in metabolic studies	775
Various	As, Ba, Co, Cr, Fe, Ga, Mo, Ni, Sb, Zn	Food	INAA	Metal migration into dry food from packing material, radiotracing; Fe and Zn were used for migration tests	776
Various	As, Be, Cd, Cr, Hg, Mn, Ni, Pb, Sn, Tl, V	Food, total diet	ICP-MS	Human exposure to metals; samples from Tarragona, Spain	777
Various		Food, water	Various	List of biological and environmental RMs	778
Various	Ca, Cr, Fe, K, Mg, Na, Zn	Foodstuffs	ETAAS	Chromium intake study	779
Various	As, Cu, Mn, Ni, Se, Zn	Foodstuffs, drinking water	ICP-MS	Daily diet intake study	780

Various	Ca, Cu, Fe, Mg, Pb, Zn	Fresh eggs	FAAS, ETAAS	Y–Pd–citric acid as CM; Zeeman effect background correction for ETAAS	781
Various	As, Cr, Cu, Fe, Mn, Ni, Pb, V, Zn	Grapes and wine	EDXRF	Multi-element analysis; samples from Krk, Adriatic island	782
Various	Cu, Fe, Mn, Zn	Hazelnuts	FAAS	Geographic origin study	783
Various	As, Hg, Pb, Sb, Se, Sn	Honey	HG-ICP-OES	MWD, HG condensation system.	784
Various	B, Ba, Ca, Cu, K, Mg, Mn, Na, P, Sr, Zn	Honey	ICP-OES	Samples from Spain; for botanical origins	785
Various	Cu, Fe, Mn, Zn	Human milk, cow's milk, baby food, rice	FAAS	Digestion procedures were compared	786
Various	Br, Ca, Cu, Fe, I, Mg, Mn, Sr, Zn	Human, cow, formula milks	ICP-MS	Fractionation, SEC	787
Various	Al, Cu, Mn, Mo, Sn	Infant formulas	ETAAS	Citric acid and HNO_3 were added, digestion at 55 °C	788
Various	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Infant formulas and pet food	AAS	Collaborative study	789
Various	Cd, Cu, Fe, Pb, Zn	Kasar cheese	FAAS, ETAAS	Ten brands of kasar cheese; samples from Turkey	790
Various	Al, Cd, Cr, Cu, Fe, Ni, Pb, Zn	Legumes, nuts	ETAAS	Samples from Spain	791
Various	As, Cd, Co, Mo, Se	Lobster	ICP-MS	Comparison of sample treatment methods	792
Various	Ca, Cd, Cr, Cu, Fe, Mg, Pb, Zn	Meat	FAAS, ETAAS	Focussed MWD and ultrasonic leaching	793
Various	Al, Ba, Cd, Cr, Cu, Mg, Mo, Ni, Pb, Sn, Zn	Milk	ICP-MS	Dietary intake of infants; MWD of six different kinds of milk	794
Various	Cd, Cr, Cu, Cs, Mo, Pb, Se, Zn	Milk, milk powder	ETAAS	Direct determination	795
Various	Cu, Fe, Ni, Pb, Zn	Mineral water	TXRF	Preconcentration with APDC; Ga as internal standard	796
Various	Cd, Cu, Hg, Pb	Mushrooms	AAS	Effect of Slovakian smelter	797
Various	Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb	Olive oil	ETAAS	Universal modifier for isoformation of analyte species and their thermal stabilization in furnace	798

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Various	Al, Ba, Bi, Ca, Cu, Mg, Mn, Na, Pb, Sn	Olive oil	FAAS, ICP-MS	Online emulsion formation; principal component analysis	799
Various	Ca, Cu, Fe, K, Mn, Zn	Onion cultivars	TRXRF	Ultrasound extraction and other procedures	800
Various	As, Cd, Cr, Hg, Pb	Packing materials	ICP-MS	Survey for packing materials	801
Various	Mn, Fe, Ni, Cu, Cd, Pb	Pasteurized milk	ETAAS	Principal component analysis for estimation of interdependences among trace metals	802
Various	Ca, Cr, Cu, Fe, Mg, Mn, Mo, Se, Zn	Pet food	ICP-OES	MW-assisted acid hydrolysis	803
Various	Cu, Fe, Mn, Zn	Porcine tissues	ICP-MS, ES-MS, ICP-OES	Optimization of extraction conditions; speciation, SEC, HPLC	804
Various	Cd, Cr, Cu, Ni, Pb	Potable water	ETAAS	Simultaneous AAS; Pd–Mg as matrix modifier	805
Various	Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, Ti, Si, Zn	Rice	XRF	Minimum sample preparation	806
Various	Cd, Hg, Pb	Rice	ETAAS, CVAAS	Rice imported into Saudi Arabia	807
Various	As, Br, Co, Fe, K, Na, Rb, Sc, Zn	Rice	INAA	Samples from Brazil	808
Various	Cd, Cu, Pb, Zn	Rice flour RM	ICP-MS, ID	MWD and ID-ICP-MS	809
Various	Cd, Cr, Cu, Fe, Mn, Pb, Sn, Zn	Sardines	FAAS	Samples from Brazil; effect of canning process	810
Various	As, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, Zn	Seafood CRMs	FAAS, ETAAS	Sample treatment techniques were compared	811
Various	As, Cd, Cr, Hg, Pb, Se,	Seafood RMs	ICP-MS, ETAAS, CVAAS	Study on candidate RMs	812
Various	Cd, Cu, Cr, Zn, Ni	Shell fish	ICP-OES	Similar results to FAAS	813
Various	Cd, Cr, Cu, Pb, Zn	Shellfish	AAS	MWD, comparable results to wet ashing, dry ashing gave low recoveries	814

Various	Al, Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Sherry wines	ICP-OES, FAAS, ETAAS	No acidification; techniques were compared	815
Various	Cd, Cu, Hg, Fe, Pb, Zn	Sparkling wines	**ETAAS**	Sample treatment by ultrasonification for degassing	816
Various	Al, Cd, Fe, Hg, Pb, Se	Spinach, cabbage	Various	Inter-laboratory study; 158 laboratories	817
Various	As, Cd, Cu, Pb, Sn	Sucrose, corn syrups, high fructose corn syrups	ICP-OES	Digestion procedures were compared	818
Various	Al, Ba, Ca, Cu, K, Mg, Mn, Zn	Tea	ICP-OES	Pattern recognition for differentiation of samples.	819
Various	Al, Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Tea infusion	FAAS	Photodecomposition; UV H_2O_2	820
Various	Ba, Ca, Co, Cu, Fe, Mg, Mn, Ni, Sr, Rb, Zn	Tea infusion	ICP-MS	Fractionation, speciation using cation exchange and SEC	821
Various	Fe, Co, Cr, Ni, Cu, Ti, Zn	Tea, herbal teas	EDXRF	Multi-element determination	822
Various	Al, Cr, Cu, Fe, Ni, Pb	Vegetable oils	ETAAS	Metallic contamination under storage was studied	823
Various	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn	Vegetable oils	ETAAS	Method development by ETAAS	824
Various	Al, Ca, Cd, Cu, Fe, Mg, Pb, Zn	Vegetables	ETAAS, FAAS	Open-vessel digestion using HCl, HNO_3 and HF.	825
Various	Cr, Ni, Pb, Sn	Vitamin E preparations	ICP-MS	Comparison of sample preparation techniques	826
Various	Al, As, Ba, Cd, Cr, Cu, Mn, Pb, Sr, U, Zn	Vodka, brandy	ICP-MS	FI; discrete injection	827
Various	Cd, Cu, Fe, Pb, Zn,	Water	FAAS	Preconcentration using fungus	828
Various	Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn	Water, beer, soft drinks	FAAS, ETAAS	Preconcentration by a liquid membrane emulsion	829
Various	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn	Wheat and rye	AAS	Swedish samples over a period of 15 years	830

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Various	As, Cd, Hg, Pb	White cheese, curd, milk	ETAAS	Trace elements in different stages from milk to white cheese	831
Various	Cd, Cr, Cu, Mn, Pb, Zn	Wine	FAAS, ETAAS	Sampling at eight stages of wine-making process	832
Various	Cd, Cr, Cu, Fe, Mn, Pb, Zn	Wine	FAAS, ETAAS	Samples from Uruguay	833
Various	As, Ca, Cu, Fe, K, Mg, Mn, Na, Zn	Wine vinegars	FAAS, HGAAS,	Classification according to fermentation rate; chemometry; samples from Spain	834
Various	Cu, Fe, Zn	Yeast	ICP-MS	LA, protein structures by MALDI-FT-ICR-MS	835
Various	Cr, Hg, I, Se, REE	Yeast	MAA	Molecular activation analysis	836
Various	Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Zn	Yoghurt, human milk	FAAS	Preconcentration methods; samples from Ethiopa	837
Various (11)		Diets, breast milk, infant formula	ICP-MS	Six-day duplicate diet study for pregnant and non-pregnant subjects	838
Various (>10)		Food	ICP-MS	High-resolution ICP-MS, ID studies	839
Various (>19)		Tea leaves	ICP-MS, ICP-OES	Speciation; separation of complexes using SEC	840
Various (>20)		Food	INAA	20–30 elements in foods and agricultural products	841
Various (11)		Coffee	ICP-OES	Characterization by metal content; chemometry	842
Various (11)		Powdered coffee,	ICP-OES	Solubilization using TMAH powdered milk	843
Various (11)		Seafood	FAAS, ETAAS, CVAAS	Sample preparation parameters were optimized	844
Various (11)		Olive oil	ICP-MS	Online emulsion formation	845
Various (11)		Wine	FAAS, FAES	Samples from Canarian Islands; chemometry; geographic origin study	846
Various (12)		Blacklip abalone ICP-MS	ETAAS, ICP-OES,	Samples from Australia	847
Various (12)		Fish	ETAAS, TXRF Vietnam	Pollution monitoring,	848
Various (12)		Honey	ICP-OES, ICP-MS	Freshly collected, extracted, ripened and sealed honey samples	849
Various (12)		Human breast milk	ICP-MS	Three digestion procedures were compared	850

Various (13)	Cereals, pulses	ICP-MS, Flame AES	Mexican food composition tables were studied	851
Various (13)	Lychee fruit	ICP-OES	Rich in Ca, Fe, Mg, Zn	852
Various (13)	Seafood	ETAAS, CVAAS	Optimization of ultrasonic bath etraction	853
various (13)	Tea	AAS, INAA, ICP-OES	Application of three methods for trace content of tea leaves and extract	854
various (13)	Wheat	FAAS, ETAAS	Origin and variety of wheat species from elemental data. Atomic spectrometry and ion chromatography were used	855
various (13)	Wheat	AAS, IC	Thirteen elements were used to assess species, origin and variety of wheat; IC and AAS	856
Various (13)	Wine	SR-TRXRF	Simple procedure, samples from Brazil	857
Various (14)	Breast milk	PIXE	Lactation and trace elements in breast milk of Nigerian women	858
Various (14)	Corn	INAA	Element distribution in five varieties of Brazilian corn	859
Various (14)	Milk, skimmed milk, whey	ICP-MS	Sector field ICP-MS	860
Various (15)	Food	ICP-OES, ICP-MS	Extraction with water soluble tertiary amine solution containing EDTA	861
Various (15)	Food	ICP-MS	HNO_3 digestion, followed by measurement; Rh and Re as internal standards; Hg memory effects were overcome	862
Various (15)	Food	ICP-MS	Method development using MWD and quadrupole ICP-MS	863
Various (15)	Human breast milk, infant formula	ICP-OES, ICP-MS	Speciation; SEC-ICP-OES or SEC-ICP-MS, binding patterns were studied; differences were found for Fe regarding two fluids	864
Various (15)	Human milk	ICP-MS	Studies to enhance sensivity and to reduce interferences	865
Various (15)	Human milk, milk formulas	ICP-MS	First report was claimed for determination of Ag, Au, Pt, Sc, Ti and V in human milk and infant formulas	866
Various (15)	Milk whey	ICP-MS	High-resolution ICP-MS	867

Table 13.5 *Continued*

Analyte	*All analytes*	*Sample*	*Technique(s)*	*Notes*	*Ref.*
Various (15)		Mussels	ETAAS, TXRF	Biomonitoring in Vietnam	868
Various (15)		Soft wheat, Durum wheat	ICP-MS	Contamination from tools used in milling and grinding; fifteen elements were released from six devices	869
Various (15)		Wines	ICP-OES	Ethanol was added to standards	870
Various (15)		Wines	ICP-OES	Classification; chemometry	871
Various (16)		Mussels	FAAS, ETAAS ICP-MS, INAA	Acid leaching; sonication; MWD for ICP-MS; solid sampling for INAA	872
Various (17)		Human milk	ICP-MS	Infant health study	873
Various (17)		Tea	ICP-OES, ICP-MS	Chemometry for classification, samples from Asia and Africa, geographic origin study	874
Various (17)		Vegetables	ICP-OES	High-pressure MWD, dry ashing	875
Various (17)		Water	ICP-MS	High-resolution ICP-MS	876
Various (18)		Human milk	ICP-MS, HGAAS	Dietary uptake from human milk was determined	877
Various (18)		Pumpkin seed oils	ICP-OES	Comparison of digestion methods	878
Various (18)		Tomatoes	ICP-OES, ICP-MS	Sector field ICP-MS	879
Various (19)		Herbs and herbal in fusions	ICP-MS, FAAS	Determination in leaves and infusions	880
Various (19)		Honey	ICP-MS	ETV-ICP-MS	881
various (19)		Milk	ICP-OES, ICP-MS	Samples were dispersed in 10 % v/v amine mixture. SRMs were used	882
Various (19)		Water	TXRF	Samples from Portugal; effect of plumbing	883
Various (19)		Wine	ICP-MS	FI; online dilution; single-standard	884
Various (21)		Food	ICP-MS	Samples from France; exposure studies	885
Various (23)		Food CRMs	ETAAS, ICP-OES	High-pressure digestion in open vessels	886
Various (23)		Food, CRMs	ICP-MS	Single microwave program for a wide range of food samples	887
Various (26)		Bottled drinking waters	ICP-MS, FAAS	Samples from Canada	888
Various (26)		Wine	ICP-MS	Comparison of methods, two laboratories	889
Various (27)		Berries, honey	SRXRF	Thin samples, in form of tablets	890

Various (27)	Wines	ICP-MS, FAAS	Samples from Czech Republic	891
Various (28)	Tea	ICP-OES, ICP-MS	Geographical origin study	892
various (29)	Human milk	ICP-OES, ICP-MS	High-pressure digestion methods using HNO_3	893
Various (29)	Wines	FAAS, ETAAS, ICP-OES	Review; comparison of techniques	894
Various (30)	Food	INAA	20–30 elements in foods and agricultural products	895
Various (30)	Food	INAA	20–30 elements in foods and agricultural products	896
Various (30)	Well water	ICP-OES	Effects of acid precipitation on results; samples from Sweden	897
Various (33)	Wines, grapes	ICP-MS	Relationship between wine composition, couor and vineyard; chemometry	898
Various (34)	Wine	ICP-MS	Samples from Canada, classification studies	899
Various (39)	Wine	ICP-MS	REEs and other elements to classify Canarian wines	900
Various (44)	Orange juice	ICP-MS	Geographic origin study; samples from Argentina, Brazil, Israel, South Africa, Morocco, Spain	901
Various (45)	Wine	ICP-MS	Determination of geographic origin; chemometry; Pb isotope ratios and rare earth element concentrations were used	902
Various (47)	Wine	ICP-MS	Effect of soil and vinification process on elemental composition; samples from Portugal	903
Various (48)	Wines	ICP-MS	Samples from England and Spain; authenticity; chemometry	904
Various (63)	Drinking water	ICP-OES, ICP-MS	Comprehensive study on samples from Ethiopia	905

References

1. *Trace Elements in Human Nutrition and Health*, WHO, Geneva, 1996.
2. F.H. Nielsen, "Other elements: Sb, Ba, B, Br, Cs, Rb. Ag, Sr, Ti, Zr, Be, Bi, Ga, Au In, Nb, Sc, Te, Ti, To, W", in *Trace Elements in Human and Animal Nutrition*, 5th edn, Vol. 2, W. Mertz (ed), Academic Press, Orlando, FL, 1986, 415.
3. F.H. Nielsen, in *Trace Minerals in Food*, K.T. Smith (ed), Marcel Dekker, New York, 1988, 357.
4. E.I. Hamilton and M.J. Minski, *Sci. Total Environ*., 1972, **1**, 375.
5. M.A. Boyle and G. Zayla, *Personal Nutrition*, 2nd edn, West Publishing Co, New York, 1992.
6. E.J. Underwood, *Trace Elements in Human and Animal Nutrition*, 3rd edn, Academic Press, New York, 1971.
7. R.A. Anderson, in *Trace Minerals in Food*, K. Smith (ed), Marcel Dekker, New York 1988, 231.
8. H. Fleck, *Macrominerals and Microminerals*, *Introduction to Nutrition*, Macmillan, New York, 1971, 172.
9. K.E. Mason, *J. Nutr*., 1979, **109**, 1979.
10. D.M. Williams, in *Clinical Biochemical and Nutritional Aspects of Trace Elements*, A.S. Prasad (ed), Alan R. Liss, New York, 1982, 277.
11. H.H. Sanstead, *Am. J. Clin. Nutr*., 1982, **32**, 908.
12. W. Mertz, *J. Am. Dietetic Assoc*., 1980. **77**, 258.
13. J. Gregory, *The Diet and Health of Adults in the United Kingdom*, HMSO, London, 1990.
14. B.S. Hetzel, *Lancet*, 1983, **ii**, 1126.
15. B.S. Hetzel, J.T. Dunn and J.T. Stunbury (eds), *The Prevention and Control of Iodine Deficiency Disorders*, Elsevier, Amsterdam, 1987.
16. J.R. Artur and G.R. Beckett, in *Selenium in Biology and Medicine*, A. Mendel (ed), Springer, Berlin, 1989, 90.
17. D.A. Koutras, in *Towards the Eradication of Endemic Goiter*, *Cretinism and Iodine Deficiency*, Dunn *et al*. (eds), Pan American Health Organization, Washington, DC, 1986, 15.
18. W.L. Rowsell, G.S. Griffith and E. Jones, *Minerals*, *Applied Science for Food Studies*, Longman Group, 1989, 97.
19. National Research Council, *Recommended Dietary Allowances*, 10th edn., National Academy of Sciences, Washington, DC, 1987.
20. K.V. Rajakopalan, *Nutr. Rev*., 1987, **45**, 321.
21. M. Anke, *Nutr. Res*., 1985, **1**(suppl.), 180.
22. M. Anke and B. Groppel, in *Trace Element Analytical Chemistry in Medicine and Biology*, P. Bratter and P. Schramel (eds), de Gruyter, Berlin, 1987, 203.
23. R.P. Hausinger, *Microbiol. Rev*., 1987, **51**, 22.
24. C.T. Walsh, *Biochemistry*, 1987, **26**, 4901.
25. M. Kirchgessner, A. Reichlmayr-Lais and R. Maier, in *Trace Elements in Man and Animals - TEMA 5*, C.F. Mills, I. Bremner and I.K. Chesters (eds), Farnham Royal, U.K., CAB International, 1985, 147.
26. N.K. Veien and M.R. Andersen, *Acta Dermato-venereologica*, 1986, **66**, 502.

27. F.H. Nielsen, in *Trace Minerals in Foods*, K.T. Smith (ed), Marcel Dekker, New York, 1988, 357.
28. G.F. Combs Jr. and S.B. Combs, *The Role of Selenium in Nutrition*, Academic Press, Orlando, FL, 1986.
29. WHO Selenium, Environmental Health Criteria, 58: A Report of the International Program on Chemical Safety, World Health Organization, Geneva, 1987.
30. E.D. Wilson, K.H. Fisher and M.E. Fuqua, *Principles of Nutrition*, 2nd edn, Wiley, New York, 1966.
31. G. Yang, *World Review of Nutrition and Diet*, 1988, **55**, 98.
32. O.A. Levander, *Ann. Rev. Nutr*., 1987, **7**, 227.
33. W.C. Willett and M.J. Stampfer, *Br. Med. J*., 1988, **297**, 573.
34. M. Mutanen, J. Kumpulainen, J. Lehto and P. Koistoinen, *Nutr. Res*., 1985, **5**, 693.
35. O.A. Levander and V.C. Morris, *Am. Clin. Nutr*., 1984, **39**, 809.
36. K. Schwarz and D.B. Milne, *Nature*, 1972, **239**, 333.
37. E.M. Carlisle, *Science*, 1972, **178**, 619.
38. National Research Council, Silicon, in *Geochemistry and the Environment. Vol. II. The Relation of Other Selected Trace Elements to Health and Disease*, National Academy of Sciences, Washington, DC, 1977, 54.
39. P. Vard and P. Koivistoii1en, *Acta Agric. Scand*., 1980, Suppl. **22**, 165.
40. J.L. Kelsay, K.M. Behall and E.S. Prather, *Am. J. Clin. Nutr*., 1979, **32**, 1876.
41. K. Schwarz, D.B. Milne and E. Vinyard, *Biochem. Biophys. Res. Commun*., 1970, **40**, 22.
42. F.H. Nielsen, *Ann. Rev. Nutr*., 1984, **4**, 21.
43. J.C. Sherlock and G.A. Smart, *Food Add. Cont*., 1984, **1**, 277.
44. J.L. Greger, in *Trace Minerals in Foods*, K.T. Smith (ed), Marcel Dekker, New York, 1988, 291.
45. B.E. Smith, *Philos. Trans. Roy. Soc. London*, *Series B*: *Biol. Sci*., 1987, **317**, 131.
46. M. Anke, in *5. Spurenelement-Symposium*: *New Trace Elements*, M. Anke *et al*. (eds), Friedrieh-SchillerUniversitat, 1986, 1266.
47. J. Somerville and B. Davies. *Am. Heart J*., 1962, **64**, 54.
48. B.R. Nechay, L.B. Nanninga, P.S.E. Nechay, J.J. Grantham, I.G. Macara, L.F. Kubena, T.D. Phillips and F.H. Nielsen, *Role of Vanadium in Biology*, Federation Proceedings, 1986, **45**, 123.
49. R. Cornelis, J. Versieck, L. Mees, J. Hoste and F. Barbier, *Bio. Trace Elem. Res*., 1981, **3**, 257.
50. F.H. Nielsen, "Vanadium," in *Trace Elements in Human and Animal Nutrition*, W. Mertz (ed), 5th edn, Vol. 1, Academic Press, San Diego, 1987, 275.
51. A.R. Byrne and L. Kosta, *Sci. Environ*., 1978, **10**, 17.
52. W.W. Evans, J. Read and D. Caughlin, *Analyst*, 1985, **110**, 873.
53. J.A.T. Pennington and J.W. Jones, *J. Am. Dietetic Assoc*., 1987, **87**, 1640.
54. D.R. Myron, T.J. Zimmerman, T.R. Shuler, L.M. Klevay, D.E. Lee, and F.H. Nielsen, *Am. J. Clin. Nutr*., 1978, **31**, 527.
55. M. Anke, U. Krause and B. Groppel, *Trace Substances Environ. Health*, 1987, **21**, 533.
56. J. Ross, R.S. Gibson and J.H. Sabry, *Trop. Geog. Med*., 1986, **38**, 246.

57. H. Fleck, *Introduction to Nutrition*, 2nd edn, Macmillan, New York, 1971, 172.
58. N.F. Krebs and K.M. Hambidge, *Am. J. Clin. Nutr.*, 1986, **43**, 288.
59. K.M. Hambidge, "Zinc," in *Trace Elements in Human and Animal Nutrition*, 5th edn, Vol. I, Mertz W (ed), Academic Press, San Diego, FL, 1987, 1.
60. "Arsenic," in *Mineral Tolerances of Domestic Animals*, National Academy of Sciences, Washington, DC, 1980, 40.
61. R.M. Parr and H. Cawley, *Dietary Intake of Minor And Trace Elements*, *A Global Survey*, IAEA, Vienna, 1990, 1.
62. Evaluation of Certain Food Additives and Contaminants, Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series, No. 776, Geneva, World Health Organization, 1989.
63. J.J. Murray, A.J. Rugg-Gunn and G.N. Jenkin, *Fluoride in Caries Prevention*, 3rd edn, Butterworth-Heinemann, London, 1991.
64. S.S. Jolly, *Fluoride*, 1976, **9**, 138.
65. N. Lakshmaiah and S.G. Srikantia, *Indian J. Med. Res.*, 1973, **65**, 543.
66. K. Krishnamachari and K**.** Kriskiiaswamy, *Lancet*, 1973, **ii**, 877.
67. D.P. Christie, *Radiology*, 1980, **138**, 85.
68. K. Kostial, D. Kello, S. Jugo, I. Rabar and T. Maljkovic, *Environ. Health Perspec*., 1978, **25**, 81.
69. Evaluation of Certain Food Additives and Contaminants, Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, World Health Organization, WHO Technical Report Series, No. 837, 1993.
70. "Cadmium," in *Toxicological Evaluation of Certain Food Additives and Contaminants*, World Health Organization, Geneva, WHO Food Additives Series, No. 24, 1989, 163.
71. C.F. Jelinek, *Assessment of Dietary Intake of Chemical Contaminants*, United Nations Environment Program, Nairobi, 1992.
72. *Guidelines for Drinking Water Quality*, 2nd edn, Vol. 1, Recommendations. World Health Organization, Geneva, 1993.
73. K. Schwarz and J. Spallholz, Growth Effects of Small Cadmium Supplements in Rats Maintained Under Trace Element Controlled Conditions, Federation Proceedings, 1976, **35**, 255.
74. M. Anke, in *Trace Element Metabolism in Man and Animals*, M. Kirchgessner (ed), Arbeitskreis fur Tierernahrung, Weihenstephan, 1978, 540.
75. S. Jugo, *Environ. Res*., 1977, **13**, 36.
76. *Methylmercury*, World Health Organization, Geneva, Environmental Health Criteria 101, 1990, 144.
77. Evaluation of Certain Food Additives and the Contaminants Mercury, Lead and Cadmium, Sixteenth report of the Expert Committee, Rome, Food and Agriculture, Organization of the United Nations, 1972 (FAO Nutrition Meetings Report Series, No. 51) World Health Organization, Geneva, WHO Technical Report Series, No. 505, 1972.
78. Evaluation of Certain Food Additives and Contaminants. Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives, World Health Organization, Geneva, WHO Technical Report Series, No. 631, 1978.

79. R.M. Parr, M. Abdulla, N.K. Aras, A.R. Byrne, C. Camara-Rica, S. Finnie, A.G. Gharib, G. Ingrao, G.V. Iyengar, F.A. Khangi, S.S. Krishnan, J. Kumpulainen, S. Liu, R. Schelenz, S. Srianqjata, J.T. Tanner and W. Wolf, Dietary Intakes of Trace Elements and Related Nutrients in Eleven Countries: Preliminary Results from an IAEA Co-ordinated Research Program, Proceedings of 7th International Symposium on Trace Elements in Man and Animals, TEMA-7. B. Momcilovic (ed), IMI, Zagreb, Yugoslavia, 1991, P. 3-3,l5.
80. "Aluminium", in *Toxicological Evaluation of Certain Food Additives and Contaminants*, Cambridge University Press, Cambridge, 1989, 113–153 (WHO Food Series, No. 24).
81. A.C. Alfrey, in *Trace Elements in Human and Animal Nutrition*, 5th edn, W. Mertz (ed), Academic Press, Orlando, FL,1986, 399.
82. M. Lotz, E. Zisman and F.C. Bartter, *N. Engl. J. Med.*, 1968, **278**, 409.
83. J.A.T. Pennington, *Food Addit. Contam*., 1988, **5**, 161.
84. P. Vinas, M. Pardo-Martinez and M. Hernandez-Cordoba, *J. AOAC Int*., 2001, **84**, 1187.
85. M.M. Vela, R.B. Toma, W. Reiboldt and A. Pierri, *Food Chem*., 1998, **63**, 235.
86. P. Vinas, N. Aguinaga, I. Lopez-Garcia and M. Hernandez-Cordoba, *J. AOAC Int*., 2002, **85**, 736.
87. M. Seruga, J. Grgic, Z. Grgic and B. Seruga, *Z. Lebensm.-Unters.- Forsch. A*, 1997, **204**, 221.
88. S. Jordan, *Analusis*, 1997, **25**, 79.
89. Z. Idiri, M. Siad, B. Kaddari, L. Omari, M. Seghir and A. Zerrouki, *J. Radioanal. Nucl. Chem*., 1993, **169**, 283.
90. P. Vinas, N. Campillo, I. Lopez-Garcia and M. Hernandez-Cordoba, *Anal. Chim. Acta*, 1997, **356**, 267.
91. S.H. Qian, G.Q. Huang and H.B. Yang Chin, *J. Anal. Chem*., 2000, **28**, 624.
92. F.F. Lopez, C. Cabrera, M.L. Lorenzo and M.C. Lopez, *Sci. Total Environ*., 2002, **300**, 69.
93. R. Ranau, J. Oehlenschlaeger and H. Steinhart, *Fresen. Z. Anal. Chem*., 1999, **364**, 599.
94. A. Krushevska and R.M. Barnes, *Analyst*, 1994, **119**, 131.
95. L. Gramiccioni, G. Ingrao, M.R. Milana, P. Santaroni and G. Tomassi, *Food Addit. Contam.*, 1996, **13**, 767.
96. S.P. Joshi, R.B. Toma, N. Medora and K. O'Connor, *Food Chem*., 2003, **83**, 383.
97. M. Nabrzyski and R. Gajewska, *Nahrung*, 1998, **42**, 109.
98. R.M. Tripathi, S. Mahapatra, R. Raghunath, A.V. Kumar and S. Sadasivan, *Sci. Total Environ.*, 2002, 299, 73.
99. F.F. Lopez, C. Cabrera, M.L. Lorenzo and M.C. Lopez, *J. Food Sci*., 2000, **65**, 206.
100. M. Yaman, *Mikrochim. Acta*, 1998, **129**, 115.
101. F.F. Lopez, C. Cabrera, M.L. Lorenzo and M.C. Lopez, *Sci. Total Environ*., 2002, **292**, 205.
102. F.F. Lopez, C. Cabrera, M.L. Lorenzo and M.C. Lopez, *Sci. Total Environ*., 2000, **257**, 191.
103. M. Plessi, D. Bertelli and A. Monzani, *J. Food Compos. Anal*., 1997, **10**, 36.

104. I. Navarro-Blasco and J.I. Alvarez-Galindo, *Food Addit. Contam*., 2003, **20**, 470.
105. M.A.Z. Arruda, M. Gallego and M. Valcarcel, *Anal. Chem*., 1993, **65**, 3331.
106. C. Maassen, S. Forster and A. Stolle, *Arch. Lebensm. Hyg*., 2003, **54**, 13.
107. E.R. Pereira, C.A. Perez, R.J. Poppi and M.A.Z. Arruda, *Spectrochim. Acta B*, 2002, **57**, 1259.
108. L.L. Sombra, M.O. Luconi, L.P. Fernandez, R.A. Olsina, M.F. Silva and L.D. Martinez, *J. Pharm. Biomed. Anal*., 2003, **30**, 1451.
109. D. Bohrer, P.C. do Nascimento, R. Binotto, E. Becker, S. Pomblum and J. Parenter, *Enter. Nutr*., 2002, **26**, 382.
110. D. Berube and D.G. Brule, *Fresen. J. Anal. Chem*., 1999, **363**, 566.
111. D. Sun, J.K. Waters and T.P. Mawhinney, *J. Agric. Food Chem*., 1997, **45**, 2115.
112. A.-K. Flaten and W. Lund, *Sci. Total Environ*., 1997, **207**, 21.
113. T.P. Flaten, C*oord. Chem. Rev.*, 2002, **228**, 385.
114. C.K. Manickum and A.A. Verbeek, *J. Anal. At. Spectrom*., 1994, **9**, 227.
115. A.A. Almeida, M.I. Cardoso and J.L.F.C. Lima, *J. Anal. At. Spectrom*., 1997, **12**, 837.
116. A.J. McKinnon and G.R. Scollary, *Talanta*, 1997, **44**, 1649**.**
117. S. Catarino, A.S. Curvelo-Garcia and R.B. de Sousa, *At. Spectrosc*., 2002, **23**, 196.
118. I. Narin, M. Tuzen and M. Soylak, *Talanta*, 2004, **63**, 411.
119. F.F. Lopez, C. Cabrera, M.L. Lorenzo and M.C. Lopez, *Sci. Total Environ*., 1998, **220**, 1.
120. S.A. Pergantis, E.M. Heithmar and T.A. Hinners, *Analyst*, 1997, **122**, 1063.
121. C. B'Hymer and J.A. Caruso, *J. Liq. Chromatogr. Relat. Technol*., 2002, **25**, 639.
122. P. Vinas, M. Pardo-Martinez and M. Hernandez-Cordoba, *J. Anal. At. Spectrom*., 1999, **14**, 1215.
123. M. Pardo-Martinez, P. Vinas, A. Fisher and S.J. Hill, *Anal. Chim. Acta*, 2001, **441**, 29.
124. P. Vinas, I. Lopez-Garcia, B. Merino-Merono, N. Campillo and M. Hernandez-Cordoba, *Chromatographia*, 2003, **57**, 611.
125. A. Martinez, A. Morales-Rubio, M.L. Cervera and M. de la Guardia, *J. Anal. At. Spectrom*., 2001, **16**, 762.
126. M. Segura, Y. Madrid and C. Camara, *J. Anal. At. Spectrom*., 1999, **14**, 131.
127. G. Concha, G. Vogler, B. Nermell and M. Vahter, *Int. Arch. Occup. Environ. Health*, 1998, **71**, 42.
128. M. Kim, *Food. Addit. Contam.*, 2004, **21**, 763.
129. N.P. Vela, D.T. Heitkemper and K.R. Stewart, *Analyst*, 2001, **126**, 1011.
130. H. Helgesen and E.H. Larsen, *Analyst*, 1998, **123(5)**, 791.
131. E.J. dos Santos and E. de Oliveira, *Braz. Arch. Biol. Technol*., 2001, **44**, 233.
132. P. Cava-Montesinos, M.L. Cervera, A. Pastor and M. de la Guardia, *Talanta*, 2003, **60**, 787.
133. S.P. Dolan, D.A. Nortrup, P.M. Bolger and S.G. Capar, *J. Agric. Food Chem*., 2003, **51(5)**, 1307.
134. A. Dudoit and S.A. Pergantis, *J. Anal. At. Spectrom*., 2001, **16**, 575.
135. C.J. Hsieh, C.H. Yen and M.S. Kuo, *Anal. Sci.*, 1999, **15**, 669.
136. L.S. Milstein, A. Essader, E.D. Pellizzari, R.A. Fernando and O. Akinbo, *Environ. Int*., 2002, **28**, 277.

137. M.L. Magnuson, J.T. Creed and C.A. Brockhoff, *J. Anal. At. Spectrom*., 1997, **12**, 689.
138. P.A. Gallagher, C.A. Schwegel, X.Y. Wei and J.T. Creed, *J. Environ. Monit.*, 2001, **3**, 371.
139. F. El-Hadri, A. Morales-Rubio and M. de la Guardia, *Talanta*, 2000, **52**, 653.
140. S. Nielsen and E.H. Hansen, *Anal. Chim. Acta*, 1997, **343**, 5.
141. R.M. Tripathi, R. Raghunath and T.M. Krishnamoorthy, *Sci. Total Environ*., 1997, **208**, 89.
142. E.H. Larsen, M. Hansen and W. Gossler, *Appl. Organomet. Chem*., 1998, **12**, 285.
143. S.S. Chen, C.C. Cheng and S.S. Chou, *J. Food Drug Anal*., 2003, **11**, 214.
144. C. Wei, W.H. Li, C. Zhang, M. Van Hulle, R. Cornelis and X.R. Zhang, *J. Agric. Food Chem*., 2003, **51**, 5176.
145. P. Fecher and G. Ruhnke, *At. Spectrosc*., 1998, **19**, 204.
146. X.C. Le, X.-F. Li, V. Lai, M. Ma, S. Yalcin and J. Feldmann, *Spectrochim. Acta B*, 1998, **53**, 899.
147. D. Juresa and M. Blanusa, *Food Addit. Contam*., 2003, **20**, 241.
148. K.L. Ackley, C. B'Hymer, K.L. Sutton and J.A. Caruso, *J. Anal. At. Spectrom*., 1999, **14**, 845.
149. M. De Gieter, M. Leermakers, R. Van Ryssen, J. Noyen, L. Goeyens and W. Baeyens, *Arch. Environ. Contam. Toxicol.*, 2002, **43**, 406.
150. K. Wrobel, B. Parker, S.S. Kannamkumarath and J.A. Caruso, *Talanta*, 2002, **58**, 899.
151. Z. Mester and P. Fodor, *J. Anal. At. Spectrom*., 1997, **12**, 363.
152. T. Lindemann, A. Prange, W. Dannecker and B. Neidhart, *Fresen. J. Anal. Chem*., 2000, **368**, 214.
153. R. Siska, J. Borszeki and E. Gegus, *Magy. Kem. Foly*., 1996, **63**, 248.
154. A.L. Moretto and S. Cadore, *Microchim. Acta*, 2004, **146**, 239.
155. P. Vinas, M. Pardo-Martinez, I. Lopez-Garcia and M. Hernandez-Cordoba, *J. Anal. At. Spectrom.*, 2001, **16**, 1202.
156. L.S. Milstein, A. Essader, C. Murrell, E.D. Pellizzari, R.A. Fernando and J.H. Raymer and O. Akinbo, *J. Agric. Food Chem*., 2003, **51**, 4180.
157. K. Julshamn, A. Maage and E.H. Larsen, *Fresen. J. Anal. Chem*., 1996, **355**, 304.
158. A. Bibak, S. Sturup, V. Haahr, P. Gundersen and V. Gundersen, *J. Agric. Food Chem*., 1999, **47**, 2678.
159. N. Salvato, *Riv. Sci. Aliment*., 1996, **25**, 197.
160. J.M. Llobett, G. Falco, C. Casas, A. Teixido and J.L. Domingo, *J. Agric. Food Chem.*, 2003, **51**, 838.
161. H. Robberecht, R. Van Cauwenbergh, D. Bosscher, R. Cornelis and H. Deelstra, *Eur. Food Res. Technol*., 2002, **214**, 27.
162. T. Roychowdhury, T. Uchino, H. Tokunaga and M. Ando, *Food Chem. Toxicol*., 2002, **40**, 1611.
163. C. Kabengera, P. Bodart, P. Hubert, L. Thunus and A. Noirfalise, *J. AOAC Int*., 2002, **85**, 122.
164. J.T. van Elteren and Z. Slejkovec, *J. Chromatogr*., *A*, 1997, **789**, 339.
165. H.J. Sternowsky, B. Moser and D. Szadkowsky, *Int. J. Hyg. Environ. Health*., 2002, **20**, 405.

166. C. Ulman, S. Gezer, O. Anal, R. Tore and U. Kirca, *Water Air Soil Pollut*., 1998, **101(1–4)**, 411.
167. A.A. Carbonell-Barrachina, P. Aracil, E. Garcia, F. Burlo and F. Martinez-Sanchez, *J. Agric. Food Chem.*, 2003, **51**, 1749.
168. J. Kirby and W. Maher, *J. Anal. At. Spectrom*., 2002, **17**, 838.
169. G. Falco, J. Gomez-Catalan, J.M. Llobet and J.L. Domingo, *Trace Elem. Electrolytes*, 2003, **20**, 120.
170. P. Cava-Montesinos, M.L. Cervera, A. Pastor and M. de la Guardia, *Talanta*, 2004, **62**, 173.
171. K.V. Nagaraja, S. Vibhakar, S.N.R. Rao and U. Rani, *Fresen. J. Anal. Chem*., 1995, **352**, 33.
172. P. Cava-Montesinos, A. de la Guardia, C. Teutsch, M.L. Cervera and M. de la Guardia, *Anal. Chim. Acta*, 2003, **493**, 195.
173. A. Krushevska, M. Kotrebai, A. Lasztity, R.M. Barnes and D. Amarasiriwardena, *Fresen. J. Anal. Chem*., 1996, **355**, 793.
174. M. Van Holderbeke, Y. Zhao, F. Vanhaecke, L. Moens, R. Dams and P. Sandra, *J. Anal. At. Spectrom.*, 1999, **14**, 2229.
175. K. Van Den Broeck, C. Vandecasteele and J.M.C. Geuns, *J. Anal. At. Spectrom*., 1997, **12**, 987.
176. M. Slekovec, W. Goessler and K.J. Irgolic, *Chem. Speciation Bioavailability*, 1999, **11**, 115.
177. A.R. Byrne, Z. Slejkovec, T. Stijve, L. Fay, W. Goessler, J. Grailer and K.J. Irgolic, *Appl. Organomet. Chem.*, 1995, **9**, 305.
178. Z. Slejkovec, J.T. van Elteren and A.R. Byrne, *Anal. Chim. Acta*, 1998, **358**, 51.
179. M.L. Cervera, J.C. Lopez and R. Montoro, *Microchem. J*., 1994, **49**, 20.
180. M. Vilano and R. Rubio, *Appl. Organomet. Chem*., 2001, **15**, 658.
181. D. Sanchez-Rodas, A. Geiszinger, J.L. Gomez-Ariza and K.A. Francesconi, *Analyst*, 2002, **127**, 60.
182. A. Chatterjee, *Talanta*, 2000, **51**, 303.
183. B.M. Hovanec, *J. Anal. At. Spectrom*., 2004, **19**, 1141.
184. S. D'Ilio, M. Alessandrelli, R. Cresti, G. Forte and S. Caroli, *Microchem. J.*, 2002, **73**, 195.
185. I. Pizarro, M. Gomez, M.A. Palacios and C. Camara, *Anal. Bioanal. Chem*., 2003, **376(1)**, 102.
186. D.T. Heitkemper, N.P. Vela, K.R. Stewart and C.S. Westphal, *J. Anal. At. Spectrom.,* 2001, **16**, 299.
187. C.G. Yuan, G.B. Jiang and B. He, *J. Anal. At. Spectrom*., 2005, **20**, 103.
188. B.S. Sheppard, D.T. Heitkemper and C.M. Gaston, *Analyst*, 1994, **119**, 1683.
189. S. Sarmani, A.K. Wood, Z. Hamzah and A.A. Majid, *J. Radioanal. Nucl. Chem*., 1993, **169**, 255.
190. O. Munoz, D. Velez and R. Montoro, *Analyst*, 1999, **124**, 4601.
191. S. McSheehy, J. Szpunar, R. Morabito and P. Quevauviller, *Trends Anal. Chem*., 2003, **22**, 191.
192. C. Santos, F. Alava-Moreno, I. Lavilla and C. Bendicho, *J. Anal. At. Spectrom*., 2000, **15**, 987.

193. M.A. Suner, V. Devesa, I. Rivas, D. Velez and R. Montoro, *J. Anal. At. Spectrom*., 2000, **15**, 1501.
194. O. Munoz, D. Velez, M.L. Cervera and R. Montoro, *J. Anal. At. Spectrom*., 1999, **14**, 1607.
195. M.C. Villa-Lojo, E. Alonso-Rodriguez, P. Lopez-Mahia, S. Muniategui-Lorenzo and D. Prada-Rodriguez, *Talanta*, 2002, **57**, 741.
196. D. Velez, N. Ybanez and R. Montoro, *J. Anal. At. Spectrom*., 1997, **12**, 91.
197. M.A. Suner, V. Devesa, O. Munoz, D. Velez and R. Montoro, *J. Anal. At. Spectrom*., 2001, **16**, 390.
198. W.H. Li, C. Wei, C. Zhang, M. Van Hulle, R. Cornelis and X.R. Zhang, *Food Chem. Toxicol.*, 2003, **41**, 1103.
199. K. Julshamn, A. Thorlacius and P. Lea, *J. AOAC Int*., 2000, **83**, 1423.
200. B.M. Gamble, P.A. Gallagher, J.A. Shoemaker, X. Wei, C.A. Schwegel and J.T. Creed, *Analyst*, 2002, **127**, 781.
201. D. Velez, N. Ybanez and R. Montoro, *J. Agric. Food Chem*., 1997, **45**, 449.
202. J.C. Lopez, C. Reija, R. Montoro, M.L Cervera and M. de la Guardia, *J. Anal. At. Spectrom.* 1994, **9**, 651.
203. M.A. Suner, V. Devesa, I. Rivas, D. Velez and R. Montoro, *J. Anal. At. Spectrom*., 2000, **15**, 1501.
204. S.A. Pergantis, K.A. Francesconi, W. Goessler and J.E. Thomas-Oates, *Anal. Chem*., 1997, **69**, 4931.
205. A. Geiszinger, S. Khokiattiwong, W. Goessler and K.A. Francesconi, *J. Mar. Biol. Assoc. U.K.*, 2002, **82**, 165.
206. J. Leblebici and M. Volkan, *J. Agric. Food Chem*., 1998, **46**, 173.
207. C.S. Dakuzaku, G.P.G. Freschi, M. de Moraes, J.A. Nobrega and J.A.G. Neto, *At. Spectrosc.*, 2001, **22**, 271.
208. C.S. Dakuzaku, G.P.G. Freschi, M. de Moraes and J.A.G. Neto, *Ecletica Quim*., 2001, **26**, 143.
209. F. Ronda, D. Sancho, C.A. Blanco, M. Gomez and P.A. Caballero, *Zuckerin dustrie*, 2002, **127**, 763.
210. I. Karadjova and T. Venelinov, *Food Addit. Contam*., 2002, **19**, 948.
211. M. Chamsaz, M.H. Arbab-Zavar and S. Nazari, *J. Anal. At. Spectrom*., 2003, **18**, 1279.
212. X.B. Yin, X.W. He, Y. Jiang and X.P. Yan, *Chem. Anal*. (*Warsaw*), 2003, **48**, 45.
213. T. Nakazato, T. Taniguchi, H. Tao, M. Tominaga and A. Miyazaki, *J. Anal. At. Spectrom*., 2000, **15**, 1546.
214. M.G.M. Alam, E.T. Snow and A. Tanaka, *Sci. Total Environ*., 2003, **308**, 83.
215. O. Munoz, O.P. Diaz, I. Leyton, N. Nunez, V. Devesa, M.A. Suner, D. Velez and R. Montoro, *J. Agric. Food Chem*., 2002, **50**, 642.
216. V.A. Resta, K.G. Fernandez, M. de Moraes and J.A.G. Neto, *At. Spectrosc*., 2002, **23**, 7.
217. P. Thomas, K. Pereira and D. Koller, *Analusis*, 1997, **25**, 19.
218. M.L. Magnuson, J.T. Creed and C.A. Brockhoff, *Analyst*, 1997, **122**, 1057.
219. M.A. Palacios, M. Gomez, C. Camara and M.A. Lopez, *Anal. Chim. Acta*, 1997, **340**, 209.

220. S. Saverwyns, X. Zhang, F. Vanhaecke, R. Cornelis, L. Moens and R. Dams, *J. Anal. At. Spectrom*., 1997, **12**, 1047.
221. B. Klaue and J.D. Blum, *Anal. Chem*., 1999, **71**, 1408.
222. J.A. Day, M.M. Montes-Bayon, A.P. Vonderheide and J.A. Caruso, *Anal. Bioanal. Chem.*, 2002, **373**, 664.
223. D. Pozebon, V.L. Dressler, A. Jose, A. Gomes Neto and J. Curtius, *Talanta*, 1998, **45**, 1167.
224. M. Ali and S.A. Tarafdar, *J. Radioanal. Nucl. Chem*., 2003, **256**, 297.
225. S.L. Anderson and S.A. Pergantis, *Talanta*, 2003, **60**, 821.
226. A. Shraim, N.C. Sekaran, C.D. Anuradha and S. Hirano, *Appl. Organomet. Chem*., 2002, **16**, 202.
227. M.M. Gonzalez, M. Gallego and M. Valcarcel, *Talanta*, 2001, **55**, 135.
228. M. Barbaste, B. Medina and J.P. Perez-Trujillo, *Food Addit. Contam*., 2003, **20**, 141.
229. S. Wangkarn and S.A. Pergantis, *J. Anal. At. Spectrom*., 1999, **14**, 657.
230. K. Tasev, I. Karadjova and T. Stafilov, *Microchim. Acta*, 2005, **149**, 55.
231. S. Wangkarn and S.A. Pergantis, *J. Anal. At. Spectrom*., 2000, **15**, 627.
232. J. Szpunar, P. Pellerin, A. Makarov, T. Doco, P. Williams and R. Lobinski, *J. Anal. At. Spectrom.*, 1999, **14**, 4639.
233. A. Krejcova and T. Cernohorsky, *Food Chem*., 2003, **82**, 303.
234. A. Simsek, D. Korkmaz, Y.S. Velioglu and O.Y. Ataman, *Food Chem*., 2003, **83**, 293.
235. T. Matsunaga, T. Ishii and H. Watanabe, *Anal. Sci*., 1996, **12**, 673.
236. D.H. Sun, J.K. Waters and T.P. Mawhinney, *J. AOAC Int*., 1997, **80**, 20.
237. E. Vassileva, C.R. Quetel and I. Petrov, *Spectrochim. Acta B*, 2003, **58**, 1553.
238. P. Vinas, M. Pardo-Martinez and M. Hernandez-Cordoba, *Anal. Chim. Acta*, 2000, **412**, 121.
239. N. Salvato, *Ann. Chim*. (*Rome*), 1997, **87**, 295.
240. R. Thalacker, A. Stelz and H. Taschan, *Brauwelt*, 1996, **136**, 1813.
241. R. Tahvonen, *Food Addit. Contam.*, 1998, **15**, 446.
242. I.M. Olsson, S. Jonsson and A. Oskarsson, *J. Environ. Monit*., 2001, **3**, 531.
243. T. Schneider, A. Haag-Kerwer, M. Maetz, M. Niecke, B. Povh, T. Rausch and A. Schussler, *Nucl. Instrum. Meth. Phys. Res*., 1999, **158**, 329.
244. A. Frkovic, M. Kras and A. Alebic-Juretic, *Bull. Environ. Contam. Toxicol*., 1997, **58**, 16.
245. N. Kugonic and H. Grcman, *Phyton-Ann. REI Bot*., 1999, **39**, 161.
246. S. Shimbo, Z.W. Zhang, T. Watanabe, H. Nakatsuka, N. Matsuda-Inoguchi, K. Higashikawa and M. Ikeda, *Sci. Total Environ*., 2001, **281**, 165.
247. Z.W. Zhang, T. Watanabe, S. Shimbo, K. Higashikawa and M. Ikeda, *Sci. Total Environ*., 1998, **220**, 137.
248. W. Sun, R. Suo and Y.K. Lu, *Anal. Sci*., 2003, **19**, 1045.
249. S. Mounicou, J. Szpunar, R. Lobinski, D. Andrey and C.J. Blake, *J. Anal. At. Spectrom*., 2002, **17**, 880.
250. S. Mounicou, J. Szpunar, D. Andrey, C. Blake and R. Lobinski, *Analyst*, 2002, **127**, 1638.

251. A. Varga, R.M. Garcinuno Martinez, G. Zaray and F. Fodor, *Spectrochim. Acta B*, 1999, **54**, 1455.
252. Z.-W. Zhang, S. Shimbo, N. Ochhi, M. Eguchi, T. Watanabe, C.-S. Moon and M. Ikeda, *Sci. Total Environ.*, 1997, **205**, 179.
253. Z. Marzec and M. Schlegel-Zawadzka, *Food Addit. Contam*., 2004, **21**, 963.
254. A. Salido and B.T. Jones, *Talanta*, 1999, **50**, 649.
255. S. Cerutti, M.F. Silva, J.A. Gasquez, R.A. Olsina and L.D. Martinez, *Spectrochim. Acta B*, 2003, **58**, 43.
256. M. Soylak, I. Narin and M. Dogan, *Anal. Lett*., 1997, **30**, 2801.
257. C.S. Moon, Z.W. Zhang, S. Shimbo, T. Watanabe, D.H. Moon, C.U. Lee, B.K. Lee, K.D. Ahn, S.H. Lee and M. Ikeda, *Environ. Res*., 1995, **71**, 46.
258. M.E. Conti, F. Cubadda and M. Carcea, *Food Addit. Contam*., 2000, **17**, 45.
259. J.M. Clarke, W.A. Norvell, F.R. Clarke and W.T. Buckley, *Can. J. Plant Sci*., 2002, **82**, 27.
260. M. Baldini, P. Stacchini, F. Cubadda, R. Miniero, P. Parodi and P. Facelli, *Food Addit. Contam.*, 2000, **17**, 679.
261. R. Tahvonen and J. Kumpulainen, *Food Addit. Contam*., 1996, **13**, 647.
262. H.M. Crews, L.M. Owen, N. Langford, S.J. Fairweather-Tait, T.E. Fox, L. Hubbard and D. Phillips, *Toxicol. Lett*., 2000, **112**, 201.
263. D.Q. Zhang, C.M. Li, L.L. Yang and H.W. Sun, *Anal. Chim. Acta*, 2000, **405**, 185.
264. S. Shimbo, Z.W. Zhang, C.S. Moon, T. Watanabe, H. Nakatsuka, N. MatsudaInoguchi, K. Higashikawa and M. Ikeda, *Int. Arch. Occup. Environ. Health*, 2000, **73**, 163.
265. T. Watanabe, Z.W. Zhang, C.S. Moon, S. Shimbo, H. Nakatsuka, N. Matsuda-Inoguchi, K. Higashikawa and M. Ikeda, *Int. Arch. Occup. Environ. Health*, 2000, **73**, 26.
266. Yu.A. Vil'pan, I.L. Grinshtein, V.A. Kopeikin and S.I. Solov'eva, *J. Anal. Chem.* (*Transl. Zh. Anal. Khim*.), 1997, **52**, 484.
267. C. Blake and B. Bourqui, *At. Spectrosc*., 1998, **19**, 6207.
268. E. Skrzydlewska, M. Balcerzak and F. Vanhaecke, *Anal. Chim. Acta*, 2003, **479**, 191.
269. R.T. White, P. Kettish and P. Kainrath, *At. Spectrosc*., 1998, **19**, 6187.
270. D. Santos, F. Barbosa, A.C. Tomazelli, F.J. Krug, J.A. Nobrega and M.A.Z. Arruda, *Anal. Bioanal. Chem*., 2002, **373**, 183.
271. C.F. Harrington, B.E. Fairman and T. Catterick, *Spectrosc. Eur*., 1997, **9(1)**, 10.
272. M.J. Baxter, H.M. Crews, P. Robb and P.R. Strutt, *Spec. Publ.–R. Soc. Chem.*, 1997, **202**, 95.
273. P.C. Aleixo, D. Santos, A.C. Tomazelli, L.A. Rufini, H. Berndt and F.J. Krug, *Anal. Chim. Acta*, 2004, **512**, 329.
274. M. Wilhelm, J. Wittsiepe, P. Schrey, U. Budde and H. Idel, *Sci. Total Environ*., 2002, **285**, 11.
275. D.Q. Zhang, C.M. Li, L.L. Yang and H.W. Sun, *Anal. Chim. Acta*, 2000, **405**, 185.
276. M. Mueller, M. Anke, H. Illing-Guenther and C. Thiel, *Food Addit. Contam.*, 1998, **15**, 135.

277. Y. Kikuchi, T. Nomiyama, N. Kumagai, T. Uemura and K. Omae, *J. Occup. Health*, 2002, **44**, 240.
278. U. Gottelt, K. Lusky, M. Stoyke and G. Henrion, *Fleischwirtschaft*, 1996, **76**, 1175.
279. P.R.M. Correia, E. Oliveira and P.V. Oliveira, *Anal. Chim. Acta*, 2000, **405**, 205.
280. C.S. Moon, J.M. Paik, C.S. Choi, D.H. Kim and M. Ikeda, *Int. Arch. Occup. Environ. Health*, 2003, **76**, 282.
281. C. Locatelli, P. Reschiglian, F. Faglioli, G. Torsi, D. Melucci and F.N. Rossi, *Ann. Chim.* (*Rome*), 1997, **87**, 397.
282. A. Hejtmankova, J. Kucerova, D. Miholova, D. Kolihova and M. Orsak, *Czech J. Anim. Sci*., 2002, **47**, 253.
283. R.F. Orsi, R.G. Wuilloud, J.C.A. de Wuilloud, R.A. Olsina and L.D. Martinez, *J. AOAC Int.*, 2002, **85**, 1410.
284. M. Kantola and T. Vartiainen, *J. Trace Elem. Med. Biol*., 2001, **15**, 11.
285. M. Roca de Togores, R. Farre and A.M. Frigola, *Sci. Total Environ*., 1999, **234**, 197.
286. R. Moreno-Rojas, P.J. Sanchez-Segarra, M.A. Amaro-Lopez and G. Zurera-Cosano, *Nahrung-Food*, 2001, **45**, 357.
287. J. Koch, I. Feldmann, N. Jakubowski and K. Niemax, *Spectrochim. Acta B*, 2002, **57**, 975.
288. U. Gottelt, G. Henrion, R. Kalaehne and M. Stoyke, *Nahrung*, 1996, **40**, 87.
289. D.S. Petterson and D.J. Harris, *Aust. J. Exp. Agric*., 1995, **35**, 403.
290. G. Zurera-Cosano, P.J. Sanchez-Segarra, M.A. Amaro-Lopez and R. Moreno-Rojas, *J. Food Addit. Contam.*, 1997, **14**, 475.
291. S.K. Dwivedi and S. Dey, *Arch. Environ. Health*, 2002, **57**, 229.
292. C.Y. Ho and S.J. Jiang, *J. Anal. At. Spectrom*., 2002, **17**, 688.
293. R. Honda, K. Tawara, M. Nishijo, H. Nakagawa, K. Tanebe and S. Saito, *Toxicology*, 2003, **186**, 255.
294. L. Racz, L. Papp, B. Prokai and Z. Kovacs, *J. Microchem*., 1996, **54**, 444.
295. C. Collin-Hansen, R.A. Andersen and E. Steinnes, *J. Phys. IV*, 2003, **107**, 311.
296. I. Lavilla, J.L. Capelo and C. Bendicho, *Fresen. J. Anal. Chem*., 1999, **363**, 3283.
297. M.C. Yebra, M.F. Enriquez and R.M. Cespon, *Talanta*, 2000, **52**, 631.
298. M.F. Enriquez-Dominguez, M.C. Yebra-Biurrun and M.P. Bermejo-Barrera, *Analyst*, 1998, **123**, 105.
299. E. Lehoczky, I. Loth and Z. Kiss, *Commun. Soil Sci. Plant Anal*., 2002, **33**, 3167.
300. D.L. Anderson, W.C. Cunningham and G.H. Alvarez, *J. Radioanal. Nucl. Chem*., 1993, **167**, 139.
301. U. Tinggi, *Food Addit. Contam*., 1998, **15**, 789.
302. I.M. Olsson and A. Oskarsson, *Analyst*, 2001, **126**, 114.
303. A. Linden, K. Andersson and A. Oskarsson, *Arch. Environ. Contam. Toxicol*., 2001, **40**, 425.
304. S.M. Sella, A.K. Avila and R.C. Campos, *Anal. Lett*., 1999, **32**, 2091.
305. K. Gunther, G. Ji and B. Kastenholz, *Fresen. J. Anal. Chem.*, 2000, **368**, 281.
306. E. Vassileva and C.R. Quetel, *Anal. Chim. Acta*, 2004, **519**, 79.

307. T. Watanabe, S. Shimbo, C.S. Moon, Z W. Zhang and M. Ikeda, *Sci. Total Environ*., 1996, **184**, 191.
308. T. Moriyama, K. Shindoh, Y. Taguchi, H. Watanabe, A. Yasui and T. Joh, *J. Food Hyg. Soc. Jpn.*, 2003, **44**, 145.
309. Q.Y. Ye, Y. Li, Y. Jiang and X.P. Yan, *J. Agric. Food Chem*., 2003, **51**, 2111.
310. C.J. Park and J.K. Suh, *J. Anal. At. Spectrom*., 1997, **12**, 573.
311. K. Baba, E. Watanabe, H. Eun and M. Ishizaka, *J. Anal. At. Spectrom*., 2003, **18**, 1485.
312. P. Bermejo-Barrera, A. Moreda-Pineiro, J. Moreda-Pineiro, T. Kauppila and A. Bermejo-Barrera, *At. Spectrosc*., 2000, **21**, 5.
313. A.R. Cruz, C.C. Vique, M.L.L. Tovar and M.C.L. Martinez, *Grasas Aceites*, 2001, **52(3–4)**, 229.
314. R. Van Cauwenbergh, D. Bosscher, H. Robberecht and H. Deelstra, *Eur. Food Res. Technol*., 2000, **212**, 13.
315. C. Kim, H.M. Chan and O. Receveur, *Food Addit. Contam*., 1998, **15**, 307.
316. S.J. Huang and S.J. Jiang, *J. Anal. At. Spectrom*., 2001, **16**, 664.
317. D.-q. Zhang, C.-m. Li, L.-l. Yang and H.-w. Sun, *J. Anal. At. Spectrom*., 1998, **13**, 1155.
318. D. Littlejohn, J.N. Egila, R.M. Gosland, U.K. Kunwar, C. Smith and X. Shan, *Anal. Chim. Acta*, 1991, **250**, 71.
319. K. Anezaki, X. Chen, T. Ogasawara, I. Nukatsuka and K. Ohzeki, *Anal. Sci*., 1998, **14**, 523.
320. H. Goenaga-Infante, M.L. Fernandez-Sanchez and A. Sanz-Medel, *J. Anal. At. Spectrom*., 1997, **12**, 1333.
321. S. Branch, S. Burke, P. Evans, B. Fairman and C.S.J. Wolff Briche, *J. Anal. At. Spectrom*., 2003, **18**, 17.
322. A.M. Chaudri, F.J. Zhao, S.P. McGrath and A.R. Crosland, *J. Environ. Qual*., 1995, **24**, 850.
323. E.J. Gawalko, T.W. Nowicki, J. Babb, R. Tkachuk and S. Wu, *J. AOAC Int*., 1997, **80**, 379.
324. G.P.G. Freschi, C.S. Dakuzaku, M. de Moraes, J.A. Nobrega and J.A.G. Neto, *Spectrochim. Acta B*, 2001, **56**, 1987.
325. R.F. Lara, R.G. Wuilloud, J.A. Salonia, R.A. Olsina, L.D. Martinez, *Fresen. J. Anal. Chem.*, 2001, **371**, 989.
326. M. Kim, *Food Addit. Contam*., 2004, **21**, 154.
327. J. Jaganathan, A.L. Reisig and S.M. Dugar, *J. Microchem*., 1997, **56**, 221.
328. M.M. Gonzalez, M. Gallego and M. Valcarcel, *J. Agric. Food Chem*., 2000, **48**, 4514.
329. B.S. Garg, J.S. Bist, R.K. Sharma and N. Bhojak, *Talanta*, 1996, **43**, 2093.
330. D. Baralkiewicz, Z. Karas and J. Siepak, *Chem. Anal.* (*Warsaw*), 1997, **42**, 691.
331. A. Carlosena, M. Gallego and M. Valcarcel, *J. Anal. At. Spectrom*., 1997, **12**, 479.
332. K. Benkhedda, H.G. Infante, E. Ivanova and F. Adams, *Fresen. J. Anal. Chem*., 2000, **368**, 288.
333. M. Gonzalez, M. Gallego and M. Valcarcel, *Talanta*, 1999, **48**, 1051.
334. C.J. Mateos, M.V. Aguilar and M.C. Martinez-Para, *J. Agric. Food Chem*., 2003, **51**, 401.

335. G.N. Flint and S. Packirisamy, *Food Addit. Contam*., 1997, **14**, 115.
336. L. Yang, Y.Z. He, W. Gan, M. Li, Q.S. Qu and X.Q. Lin, *Talanta*, 2001, **55**, 271.
337. Y.Z. He, M.L. Cervera, A. Pastor and M. de la Guardia, *Anal. Chim. Acta*, 2001, **447**, 135.
338. E. Lendinez, M.L. Lorenzo, C. Cabrera and M.C. Lopez, *Sci. Total Environ*., 2001, **278**, 183.
339. F. Cubadda, S. Giovannangeli, F. Iosi, A. Raggi and P. Stacchini, *Food Chem.*, 2003, **81**, 463.
340. M. Accominotti, M. Bost, P. Haudrechy, B. Mantout, P.J. Cunat, F. Comet, C. Mouterde, F. Plantard, P. Chambon and J.J. Vallon, *Contact Dermatitis*, 1998, **38**, 305.
341. U. Tinggi, C. Reilly and C. Patterson, *Food Chem*., 1997, **60**, 123.
342. M.S. Bratakos, E.S. Lazos and S.M. Bratakos, *Sci. Total Environ*., 2002, **290**, 47.
343. P. Tiglea and J. Lichtig, *Anal. Lett*., 2000, **33**, 1615.
344. E. Garcia, C. Cabrera, M.L. Lorenzo and M.C. Lopez, *Sci. Total Environ*., 2000, **247**, 51.
345. M.E. Soares, M.L. Bastos and M. Ferreira, *J. AOAC Int*., 2000, **83**, 220.
346. R.E.L. de Ruiz, R.A. Olsina and A.N. Masi, *X-Ray Spectrom*., 2002, **31**, 150.
347. C.G. Bruhn, J.Y. Neira, M.I. Guzman, M.M. Darder and J.A. Nobrega, *Fresen. J. Anal. Chem*., 1999, **364**, 273.
348. S.P. Quinaia and J.A. Nobrega, *Quim. Nova*, 2000, **23**, 185.
349. G.M. Wuilloud, R.G. Wuilloud, J.C.A. de Wuilloud, R.A. Olsina and L.D. Martinez, *J. Pharm. Biomed. Anal*., 2003, **31**, 117.
350. J. Lameiras, M.E. Soares, M. Lourdes Bastos and M. Ferreira, *Analyst*, 1998, **123**, 2091.
351. F. Sacher, B. Raue, J. Klinger and H.J. Brauch, *Int. J. Environ. Anal. Chem*., 1999, **74**, 191.
352. A. Zybin, G. Schaldach, H. Berndt and K. Niemax, *Anal. Chem*., 1998, **70(23)**, 5093.
353. A.C. Sahayam, *Anal. Bioanal. Chem*., 2002, **372**, 840.
354. J. Posta, A. Gaspar, R. Toth and L. Ombodi, *Fresen. J. Anal. Chem*., 1996, **355**, 719.
355. A.C. Sahayam, J. Arunachalam and S. Gangadharan, *Can. J. Anal. Sci. Spectrosc*., 1998, **43**, 4.
356. M.M. Gonzalez, M. Gallego, M. Valcarcel and G. Gomez-Cardenas, *J. AOAC Int*., 2001, **84**, 1914.
357. E. Lendinez, M.C. Lopez, C. Cabrera and M.L. Lorenzo, *J. AOAC Int*., 1998, **81**, 1043.
358. C. Cabrera-Vique, P.-L. Teissedre, M.-T. Cabanis and J.-C. Cabanis, *J. Agric. Food Chem*., 1997, **45**, 1808.
359. E.A. Hernandez-Caraballo, R.M. Avila-Gomez, T. Capote, F. Rivas and A.G. Perez, *Talanta*, 2003, **60**, 1259.
360. T. Capote, L.M. Marco, J. Alvarado and E.D. Greaves, *Spectrochim. Acta B*, 1999, **54**, 1463.
361. J.M. Anzano, M. Paz Martinez-Garbayo, M.A. Bellarra and J.R. Castillo, *J. Anal. At. Spectrom*. 1994, **9**, 125.

362. D. Bellido-Milla, A. Onate-Jaen, J.M. Palacios-Santander, D. Palacios-Tejero and M.P. Hernandez-Artiga, *Microchim. Acta*, *2004*, **144**, 183.
363. S.M.V. Fernandes and A.O.S.S. Rangel, *J. AOAC Int*., 1998, **81**, 645.
364. P. Vinas, N. Campillo, I.L. Garcia and M.H. Cordoba, *J. Agric. Food Chem*., 1993, **41**, 2024.
365. E.M.D. Flores, A.B. da Costa, J.S. Barin, V.L. Dressler, J.N.G. Paniz and A.F. Martins, *Spectrochim. Acta B*, 2001, **56**, 1875 .
366. C. Lelievre, D. Hennequin, J.F. Lequerler and D. Barillier, *At. Spectrosc*., 2000, **21**, 23.
367. D. Hennequin, C. Lelievre and D. Barillier, *Analusis*, 1998, **26**, M25.
368. C. Lelievre, D. Hennequin, D. Barillier and F. Lemasson, *Analusis*, 1996, **24**, 39.
369. F. Dorey, D. Barillier, F. Bosque, V. Paraire and J.F. Le Querler, *Analusis*, 1993, **21**, 49.
370. T. Kawada, Y.Z. Lee, S. Suzuki and I.F. Rivai, *J. Trace Elem. Med. Biol*., 2002, **16**, 179.
371. S. Nikdel, D.G. MacKeller and R.J. Rezaaiyan, *J. Agric. Food Chem*., 1991, **39**, 1773.
372. T.J. Gluodenis, Jr. and J.F. Tyson, *J. Anal. At. Spectrom*., 1992, **7**, 301.
373. M.M. Gomez, T. Gasparic, M.A. Palacios and C. Camara, *Anal. Chim. Acta*, 1998, **374**, 241.
374. P. Vinas, M. Pardo-Martinez, N. Campillo and M. Hernandez-Cordoba, *J. AOAC Int*., 1999, **82**, 2368.
375. R. Eiden, R. Falter, B. Augustin-Castro and H.F. Schoeler, *Fresen. J. Anal. Chem*., 1997, **357**, 439.
376. N. Kodama, Y. Hitachi, H. Takeyama, T. Fuchi and M. Nishimuta, *Maguneshumu* (*Kyoto*), 1995, **14**, 139.
377. M.T. Vidal, M.C. Pascual-Marti, A. Salvador and C. Llabata, *Microchem. J*., 2002, **72**, 221.
378. K.A. Skibniewska, *Food Addit. Contam*., 2002, **19**, 969.
379. M. Murillo, Z. Benzo, E. Marcano, C. Gomez, A. Garaboto and C. Marin, *J. Anal. At. Spectrom*., 1999, **14**, 815.
380. L.B. Allen, P.H. Siitonen and H.C. Thompson Jr., *J. Am. Oil Chem. Soc*., 1998, **75**, 477.
381. D. Bosscher, R. Van Cauwenbergh, H. Robberecht, M. Van Caillie-Bertrand and H. Deelstra, *Eur. Food Res. Technol*., 2002, **215**, 275.
382. Y.H. Pang, D.L. MacIntosh and P.B. Ryant, *J. Nutr.*, 2001, **131**, 2171.
383. S. Shang and W. Hong, *Talanta*, 1997, **44**, 269.
384. N.J. Miller-Ihli, *J. Food Compos. Anal*., 1996, **9**, 301.
385. E.C. Ferreira, S. Rodrigues, M.M.C. Ferreira, J.A. Nobrega and A.R.A. Nogueira, *Ecletica Quim*., 2002, **27**, 77.
386. N. Amin, S. Kaneco, T. Suzuki and K. Ohta, *Microchem. J*., 2003, **74**, 181.
387. S.F. Dong and Z.G. Zhu, *Spectrosc. Spectr. Anal*., 2002, **22**, 478.
388. M.D. Silvestre, M.J. Lagarda, R. Farre, C. Martinez-Costa, J. Brines, A. Molina and G. Clemente, *Biol. Trace Elem. Res*., 2000, **76**, 217.
389. D. Silvestre, C. Martinez-Costa, M.J. Lagarda, J. Brines, R. Farre and G. Clemente, *Biol. Trace Elem. Res*., 2001, **80**, 1.

390. N. Campillo, P. Vinas, I. Lopez-Garcia and M. Hernandez-Cordoba, *Talanta*, 1998, **46**, 615.
391. K.M. Hua, M. Kay and H.E. Indyk, *Food Chem*., 2000, **68**, 463.
392. C.R. Nogueira, C. Colli and O.M.S. Amancio, *Food Chem*., 2005, **90**, 779.
393. F. Uzondo, M.T. Vidal and M. De La Guardia, *Analusis*, 1991, **19**, 136.
394. J. Jaganathan and S.M. Dugar, *At. Spectrosc*., 1997, **18**, 156.
395. Z. Benzo, E. Marcano, C. Gomez, F. Ruiz, J. Salas, M. Quintal, A. Garaboto and M. Murillo, *J. AOAC Int*., 2002, **85**, 967.
396. N. Carrion, A. Itriago, M. Murillo, E. Eljuri and A. Femandez, *J. Anal. At. Spectrom*., 1994, **9**, 205.
397. P. Bermejo Barrera, R. Dominguez Gonzalez and A. Bermejo Barrera, *Fresen. J. Anal. Chem.*, 1997, **357**, 457.
398. O. Abollino, M. Aceto, M.C. Bruzzoniti, E. Mentasti and C. Sarzanini, *Anal. Chim. Acta*, 1998, **375**, 299.
399. E.H. Soufleros, A.S. Mygdalia and P. Natskoulis, *Food Chem*., 2004, **86**, 625.
400. A. Moreno-Cid and M.C. Yebra, *Spectrochim. Acta B*, 2002, **57**, 967.
401. V. Carbonel, A.R. Mauri, A. Salvador and M. De la Quardia, *J. Anal. At. Spectrom.*, 1991, **6**, 581.
402. S.P. Fili, E. Oliveira and P.V. Oliveira, *J. Braz. Chem. Soc*., 2003, **14**, 435.
403. J.M. Anzano and P. Gonzalez, *Microchem. J*., 2000, **64**, 141.
404. E. Almeida, V.F. Nascimento, E.P.E. Valencia and R. Silva, *J. Radioanal. Nucl. Chem*., 2002, **252**, 541.
405. P.J. Sanchez-Segarra, M. Garcia-Martinez, M.J. Gordillo-Otero, A. Diaz-Valverde, M.A. Amaro-Lopez and R. Moreno-Rojas, *Food Chem*., 2000, **71**, 85.
406. D. Joseph, M. Lal, H.N. Bajpai and P.K. Mathur, *J. Food Sci. Technol*., 1999, **36**, 264.
407. A. De Leonardis, V. Macciola and M. De Felice, *Int. J. Food Sci. Technol*., 2000, **35**, 371.
408. M. Pankotai Gilinger and P.J. Fodor, *Environ. Sci. Health B*, 1996, **B31**, 621.
409. M. de la Guardia, V. Carbonell, A. Morales-Rubio and A. Salvador, *Talanta*, 1993, **40**, 1609.
410. J.A. Sweileh, *Microchem. J*., 2000, **65**, 87.
411. A.N. Anthemidis, G.A. Zachariadis and J.A. Stratis, *Talanta*, 2001, **54**, 935.
412. E. Kenduzler and A.R. Turker, *Anal. Chim. Acta*, 2003, **480**, 259.
413. R.C.C. Costa, M.I. Cardoso and A.N. Araujo, *Am. J. Enol. Vitic*., 2000, **51**, 131.
414. S. Frias, J.P. Trujillo, E.M. Pena and J.E. Conde, *Eur. Food Res. Technol*., 2001, **213**, 145.
415. G. Schwedt, U. Blum, and C. Wiese, *CLB Chem. Labor Biotech*., 1997, **48**, 436.
416. J.S. Bian, F. Shan and Y.Q. Li, *Spectrosc. Spectr. Anal*., 2000, **20**, 381.
417. T. Murakami, N. Narita, H. Nagakagi, T. Shibata and C. Robinson, *Caries Res.*, 2002, **36**, 386.
418. B.B. Wang, B.S. Zheng, C. Zhai, G.Q. Yu and X.J. Liu, *Environ. Int*., 2004, **30**, 1067.
419. A.M. Franco, S. Martignon, A. Saldarriaga, M.C. Gonzales, M.I. Arbelaez, A. Ocampo, L.M. Luna, E.A. Martinez-Mier and A.E. Villa, *Community Dent. Oral*, 2005, **33**, 1.

420. T.A. Marshall, S.M. Levy, J.J. Warren, B. Broffitt, J.M. Eichenberger-Gilmore and P.J. Stumbo, *J. Am. Coll. Nutr*., 2004, **23**, 108.
421. F.V. Zohouri, A.J. Rugg-Gunn, E.S. Fletcher, A.F. Hackett, P.J. Moynihan and J.C. Mathers, *Brit. Dent. J*., 2004, **196**, 547.
422. J. Kaipio, S. Nayha and V. Valtonen, *Eur. J. Cardiovasc. Rev. Rehal*., 2004, **11**, 56.
423. N.J. Miller-Ihli, P.R. Pehrson, R.L. Cutrifelli and J.M. Holden, *J. Food Compos. Anal*., 2003, **16**, 621.
424. M. Gomez, I. Rodriguez, C. Camara and M.A. Palacios, *Analyst*, 1990, **115**, 553.
425. P. Ampan, S. Lapanantnoppakhun, P. Sooksamiti, J. Jakmunee, S.K. Hartwell, S. Jayasvati, G.D. Christian and K. Grudpan, *Talanta*, 2002, **58**, 1327.
426. G. Doner and A. Ege, *Anal. Chim. Acta*, 2004, **520**, 217.
427. P. Bermejo, E. Pena, R. Dominguez, A. Bermejo, J.M. Fraga and J.A. Cocho, *Talanta*, 2000, **50**, 1211.
428. G. Doner and S. Akman, *Anal. Lett*., 2000, **33**, 3333.
429. I. Lestienne, C. Icard-Verniere, C. Mouquet, C. Picq and S. Treche, *Food Chem*., 2005, **89**, 421.
430. S.M. Chan, E.A.S. Nelson, S.S.F. Leung, P.C.K. Cheung and C.Y. Li, *Eur. J. Clin. Nutr*., 2000, **54**, 797.
431. J. Park and H.C. Brittin, *J. Food Qual.*, 2000, **23**, 205.
432. F.V. Silva, G.S. Lopes, J.A. Nobrega, G.B. Souza and A.R.R.A. Nogueira, *Spectrochim. Acta B*, 2001, **56**, 1909.
433. P. Bermejo, R. Dominguez and A. Bermejo, *Talanta*, 1997, **45**, 325.
434. H. Grajeta, A. Prescha and J. Biernat, *Nahrung-Food*, 2002, **46**, 7.
435. M. Ake, E. Atindehou, A.K. Malan and B. Mandrou, *Sci. Des Aliments*, 1999, **19**, 593.
436. A.M.A. Barelli, J.V. Visentainer, M. Matsushita and N. Evelazio de Souza, *Arq. Biol. Technol.*, 1995, **38**, 1199.
437. X.N. Dong, Y. Nakaguchi and K. Hiraki, *Anal. Sci*., 1998, **14**, 785.
438. R.E. Serfass and M.B. Reddy, *J. Nutr*., 2003, **133**, 449.
439. D. Bosscher, M. Van Caillie-Bertrand, H. Robberecht, K. Van Dyck, R. Van Cauwenbergh and H. Deelstra, *J. Pediatr. Gastroenterol. Nutr.*, 2001, **32**, 54.
440. P. Bermejo, E.M. Pena, R. Dominguez, A. Bermejo, J.A. Cocho and J.M. Fraga, *Food Chem.*, 2002, **77**, 361.
441. D. Bosscher, M. Van Caillie-Bertand, K. Van Dyck, H. Robberecht, R. Van Cauwenbergh and H. Deelstra, *J. Pediatr. Gastroenterol. Nutr.*, 2000, **30**, 373.
442. J. Carlos de Andrade, F.C. Strong, III and N.J. Martin, *Talanta*, 1990, **37**, 711.
443. C.F. Harrington, S. Elahi, S.A. Merson and P. Ponnampalavanar, *Anal. Chem.*, 2001, **73**, 4422.
444. A. Moreno-Cid, M.C. Yebra, S. Cancela and R.M. Cespon, *Anal. Bioanal. Chem.*, 2003, **377**, 730.
445. B. Benitez, A. Archile, L. Rangel, M. Bracho, M. Hernandez and E. Marquez, *Arch. Latinoam. Nutr.*, 2002, **52**, 307.
446. L. Perring and D. Andrey, *J. Agric. Food Chem*., 2003, **51**, 4207.
447. M.A. Belarra, M.P. Martinez-Garbayo and J.M. Anzano, *J.R. Castillo Anal. Sci*., 1996, **12**, 483.

448. M.M. Silva, M.G.R. Vale, I.C.F. Damin, B. Welz, M. Mandaji and J.P. Fett, *Anal. Bioanal. Chem.*, 2003, **377**, 165.
449. P. Garcia, C. Romero, M. Brenes and A. Garrido, *J. Agric. Food Chem.*, 2002, **50**, 3654.
450. P. Vinas, N. Campillo, I. Lopez-Garcia and M. Hernandez-Cordoba, *Anal. Chim. Acta*, 1993, **283**, 393.
451. E. Kenduzler and A.R. Turker, *Anal. Sci*., 2002, **18(8)**, 917.
452. R.C.D. Costa and A.N. Araujo, *Anal. Chim. Acta*, 2001, **438**, 227.
453. E.K. Paleologos, D.L. Giokas, S.M. Tzouwara-Karayanni and M.I. Karayannis, *Anal. Chim. Acta*, 2002, **458**, 241.
454. M. Olalla, M.C. Gonzalez, C. Cabrera and M.C. Lopez, *J. AOAC Int*., 2000, **83**, 189.
455. M. Olalla, M.C. Gonzalez, C. Cabrera and M.C. Lopez, *J. AOAC Int*., 2000, **83**, 189.
456. P. Vinas, M. Pardo-Martinez, I. Lopez-Garcia and M. Hernandez-Cordoba, *J. Anal. At. Spectrom*., 2001, **16**, 633.
457. I. Gelaude, R. Dams, M. Resano, F. Vanhaecke and L. Moens, *Anal. Chem*., 2002, **74**, 3833.
458. M.S. da Rocha, A.B. Soldado, E. Blanco and A. Sanz-Medel, *J. Anal. At. Spectrom*., 2001, **16**, 951.
459. E. Ramalhosa, S.R. Segade, E. Pereira, C. Vale and A.R. Duarte, *Analyst*, 2001, **126(9)**, 1583.
460. H. Drexler and K.-H. Schaller, *Environ. Res*., 1998, **77**, 2124.
461. M. Logar, M. Horvat, I. Falnoga and V. Stibilj, *Fresen. J. Anal. Chem*., 2000, **366**, 453.
462. A. Manova, E. Beinrohr and L. Jurica, *Chem. Pap.-Chem. Zvesti*, 2003, **57**, 197.
463. J.C.A. de Wuilloud, R.G. Wuilloud, J.A. Gasquez, R.A. Olsina and L.D. Martinez, *At. Spectrosc*., 2001, **22**, 398.
464. A.I. Cabanero, Y. Madrid and C. Camara, *Anal. Chim. Acta*, 2004, **526**, 51.
465. A.I C. Ortiz, Y.M. Albarran and C.C. Rica, *J. Anal. At. Spectrom*., 2002, **17**, 1595.
466. R. Rai, W. Maher and F. Kirkowa, *J. Anal. At. Spectrom*., 2002, **17**, 1560.
467. L. Yang, Z. Mester and R.E. Sturgeon, *J. Anal. At. Spectrom*., 2003, **18**, 431.
468. L. Yang, V. Colombini, P. Maxwell, Z. Mester and R.E. Sturgeon, *J. Chromatogr. A*, 2003, **1011**, 135.
469. X.P. Yan, Y. Li and Y. Jiang, *Anal. Chem*., 2003, **75**, 2251.
470. R. Scerbo and C. Barghigiani, *Environ. Technol*., 1998, **19**, 339.
471. C. Gerbersmann, M. Heisterkamp, F.C. Adams and J.C.A.C. Broekaert, *Anal. Chim. Acta*, 1997, **350**, 273.
472. A.V. Yallouz, R. Calixto de Campos and S. Paciornik, *Fresen. J. Anal. Chem*., 2000, **366**, 461.
473. Q. Tu, J. Qian and W. Frech, *J. Anal. At. Spectrom*., 2000, **15**, 1583.
471. Y. Cai, G. Tang, R. Jaffe and T. Jones, *Int. J. Environ. Anal. Chem*., 1997, **68**, 331.
475. L. Aduna de Paz, A. Alegria, R. Barbera, R. Farre and M.J. Lagarda, *Food Chem*., 1997, **58**, 169.
476. H.B. Swan, *Hewlett-Packard Peak*, 1996, 2.

477. F. Ubillus, A. Alegria, R. Barbera, R. Farre and M.J. Lagarda, *Food Chem.*, 2000, **71**, 529.
478. L.D.N. dos Santos, R.C.S. Muller, J.E.D. Sarkis, C.N. Alves, E.D. Brabo, E.D. Santos and M.H.D. Bentes, *Sci. Total Environ.*, 2000, **261**, 1.
479. J.N. Morgan, M.R. Berry and R.L. Graves, *J. Exposure Anal. Environ. Epidemiol.,* 1997, **7**, 119.
480. I. Rodriguez Pereiro, A. Wasik and R. Lobinski, *J. Anal. At. Spectrom.*, 1998, **13**, 743.
481. A. Bortoli, M. Gerotto, M. Marchiori, H. Mantau and A. Rehnert, *Mikrochim. Acta*, 1995, **119**, 305.
482. O.C. Vaidya and R.T.T. Rantala, *Int. J. Environ. Anal. Chem.*, 1996, **63**, 179.
483. M.L. Relon, *Philipp. J. Sci.*, 1996, **125**, 305.
484. W. Holak, *J. Assoc. Off. Anal. Chem.*, 1989, **72**, 926.
485. M.M. Storelli, R. Giacominelli-Stuffler, A. Storelli, R. D'Addabbo, C. Palermo and G.O. Marcotrigiano, *Food Addit. Contam.*, 2003, **20**, 1114.
486. M.-J. Liaw, S.-J. Jiang and Y.-C. Li, *Spectrochim. Acta B*, 1997, **52B**, 779.
487. E.C.O. Santos, V.M. Camara, I.M. Jesus, E.S. Brabo, E.C.B. Loureiro, A.F.S. Mascarenhas, K.F. Fayal, G.C. Sa, F.E.S. Sagica, M.O. Lima, H. Higuchi and I.M. Silveira, *Environ. Res.*, 2002, **90**, 6.
488. G.R. Boaventura, A.C. Barbosa and G.A. East, *Biol. Trace Elem. Res.*, 1997, **60**, 153.
489. J.A. Moreton and H.T. Delves, *J. Anal. At. Spectrom.*, 1998, **13**, 659.
490. P. Vinas, M. Pardo-Martinez, I. Lopez-Garcia and M. Hernandez-Cordoba, *J. Agric. Food Chem.*, 2002, **50**, 949.
491. L.L. Yang, D.Q. Zhang and Q.X. Zhou, *Anal. Sci.*, 2002, **18**, 811.
492. M.R. Madson and R.D. Thompson, *J. AOAC Int.*, 1998, **81**, 808.
493. R.W. Dabeka, P. Bradley and A.D. McKenzie, *J. AOAC Int.*, 2002, **85**, 1136.
494. Y. Li, Y. Jiang, X.P. Yan and Z.M. Ni, *Environ. Sci. Technol.*, 2002, **36**, 4886.
495. R. Jedrzejczak, *Food Addit. Contam.*, 2002, **19**, 996.
496. W.L. Clevenger, B.W. Smith and J.D. Winefordner, *Crit. Rev. Anal. Chem.*, 1997, **27**, 1.
497. R. Nakagawa and Y. Yumita, *Kankyo Kagaku Kenkyu Hokoku* (*Chiba Daigaku*), 1997, **22**, 13.
498. C. Gundacker, B. Pietschnig, K.J. Wittmann, A. Lischka, H. Salzer, L. Hohenauer and E. Schuster, *Pediatrics,* 2002, **110**, 873.
499. J. Falandysz and L. Bielawski, *Pol. J. Environ. Stud.*, 2001, **10**, 67.
500. J. Falandysz, M. Gucia, A. Brzostowski, M. Kawano, L. Bielawski, A. Frankowska and B. Wyrzykowska, *Food Addit. Contam.*, 2003, **20**, 247.
501. J. Falandysz, M. Kawano, A. Swieczkowski, A. Brzostowski and M. Dadej, *Food Chem.,* 2003, **81**, 21.
502. M.K. Donais, R. Saraswati, E. Mackey, R. Demiralp, A. Porter, M. Vangel, M., Levenson, V. Mandic, S. Azenard, M. Horvat, K. May, H. Emons and S.A. Wise, *Fresen. J. Anal. Chem.*, 1997, **358**, 424.
503. M. Horvat, L. Liang, S. Azemard, V. Mandic, J.-P Villeneuve and M. Coquery, *Fresen. J. Anal. Chem.*, 1997, **358**, 411.
504. S. Rio Segade and C. Bendicho, *Ecotoxicol. Environ. Saf.*, 1999, **42**, 245.

505. C. Dietz, Y. Madrid and C. Camara, *J. Anal. At. Spectrom*., 2001, **16**, 1397.
506. E. Fatemian, J. Allibone and P.J. Walker, *Analyst*, 1999, **124**, 1233.
507. J. Allibone, E. Fatemian and P.J. Walker, *J. Anal. At. Spectrom*., 1999, **14**, 235.
508. H.-C. Wang, Y.-C. Hwang, C.-J. Hsieh and M.-S. Kuo, *Anal. Sci*., 1998, **14**, 983.
509. L.N. Liang, G.B. Jiang, J.F. Liu and J.T. Hu, *Anal. Chim. Acta*, 2003, **477**, 131.
510. K. Julshamn and J. Brenna, *J. AOAC Int*., 2002, **85**, 626.
511. S. Landi, F. Fagioll, C. Locatelli and R. Vecchietti, *Analyst*, 1990, **115**,173.
512. J.C.A. de Wuilloud, R.G. Wuilloud, M.F. Silva, R.A. Olsina and L.D. Martinez, *Spectrochim. Acta B*, 2002, **57**, 365.
513. J.L. Mann, S.E. Long and W.R. Kelly, *J. Anal. At. Spectrom*., 2003, **18**, 1293.
514. J.L. Manzoori, M.H. Sorouraddin and A.M.H. Shabani, *J. Anal. At. Spectrom*., 1998, **13**, 305.
515. J. Falandysz, K. Lipka, M. Gucia, M. Kawano, K. Strumnik and K. Kannan, *Environ. Int*., 2002, **28**, 421.
516. S. Frias, C. Diaz, J.E. Conde and J.P.P. Trujillo, *Food Addit. Contam*., 2003, **20**, 237.
517. I. Karadjova, S. Arpadjan, J. Cvetkovic and T. Stafilov, *Microchim. Acta*, 2004, **147**, 39.
518. P. Herbst, M. Netzer, K. Heili and F. Brandion, *Mitt. Klosterneuburg*, 1998, **48**, 149.
519. T. Perez-Corona, Y. Madrid-Albarran, C. Camara and E. Beceiro, *Spectrochim. Acta B*, 1998, **53**, 321.
520. M. Leiterer, D. Truckenbrodt and K. Franke, *Eur. Food Res. Technol*., 2001, **213**, 150.
521. G. Jahreis, W. Hausmann, G. Kiessling, K. Franke and M. Leiterer, *Exp. Clin. Endocrinol. Diabet*., 2001, **109**, 163.
522. B.R. Norman and G.V. Iyengar, *Fresen. J. Anal. Chem*., 1994, **348**, 430.
523. E.H. Larsen and M.B. Ludwigsen, *J. Anal. At. Spectrom*., 1997, **12**, 435.
524. G. Radlinger and K.G. Heumann, *Anal. Chem*., 1998, **70**, 2221.
525. P.A. Fecher, I. Goldmann and A. Nagengast, *J. Anal. At. Spectrom*., 1998, **13**, 977.
526. R.R. Rao and A. Chatt, *Analyst*, 1993, **118**, 1247.
527. M. Rose, P. Miller, M. Baxter, G. Appleton, H. Crews and M. Croasdale, *J. Environ. Monit*., 2001, **3**, 361.
528. M. Haldimann, A. Eastgate and B. Zimmerli, *Analyst*, 2000, **125**, 1977.
529. P. Bermejo-Barrera, R.M. Anllo-Sendin, M. Aboal-Somoza and A. Bermejo-Barrera, *Mikrochim. Acta*, 1999, **131**, 145.
530. P. Bermejo-Barrera, L.M. Fernandez-Sanchez, M. Aboal-Somoza, R.M. Anllo-Sendin and A. Bermejo-Barrera, *Microchem. J*., 2001, **69**, 205.
531. J. Naozuka, M. da Veiga, P.V. Oliveira and E. de Oliveira, *J. Anal. At. Spectrom*., 2003, **18**, 917.
532. M. Resano, E. Garcia-Ruiz, L. Moens and F. Vanhaecke, *J. Anal. At. Spectrom*., 2005, **20**, 81.
533. P. Bermejo-Barrera, M. Aboal-Somoza, A. Bermejo-Barrera, M.L. Cervera and M. de la Guardia, *J. Anal. At. Spectrom*., 2001, **16**, 382.
534. L.B. Rasmussen, E.H. Larsen and L. Ovesen, *Eur. J. Clin. Nutr*., 2000, **54**, 57.
535. L. Dahl, J.A. Opsahl, H.M. Meltzer and K. Julshamn, *Br. J. Nutr*., 2003, **90**, 679.

536. L. Fernandez Sanchez and J. Szpunar, *J. Anal. At. Spectrom.*, 1999, **14**, 1697.
537. L.F. Sanchez and J. Szpunar, *J. Anal. At. Spectrom.*, 1999, **14**, 1697.
538. H. Baumann, *J. Fresen. Anal. Chem.*, 1990, **338**, 809.
539. Y. Gelinas, A. Krushevska and R.M. Barnes, *Anal. Chem.*, 1998, **70**, 1021.
540. F.Y. Iskander, *J. Assoc. Off. Anal. Chem.*, 1989, **72**, 498.
541. K. Julshamn, L. Dahl and K. Eckhoff, *J. AOAC Int.*, 2001, **84**, 1976.
542. M.C. Yebra and R.M. Cespon, *Anal. Chim. Acta*, 2000, **405**, 191.
543. P. Hayumbu, M.B. Zaman, P.C. Chigali, S. Munsanje and P. Mulenga, *Int. J. Biochemiphys.*, 1995, **4**, 2.
544. R.M. Parr, H. Kawamura and G.V. Iyengar, *Biol. Trace Elem. Res.*, 1999, **71–72**, 5.
545. X.P. Wang and C.Y. Wang, *Spectrosc. Spectr. Anal.*, 2002, **22**, 163.
546. M.N. Amin, S. Kaneco, T. Suzuki, Y. Taniguchi and K. Ohta, *Anal. Bioanal. Chem.*, 2002, **373**, 205.
547. R.J.N. Bettencourt da Silva, M.F.G.F.C. Camoes and J. Seabra e Barros, *Accred. Qual. Assur.*, 1998, **3**, 155.
548. G. Weber and P. Konieczynski, *Anal. Bioanal. Chem.*, 2003, **375**, 1067.
549. M.C. Yebra and A. Moreno-Cid, *Anal. Chim. Acta*, 2003, **477**, 149.
550. Y. Ozdemir and S. Gucer, *Food Chem.*, 1998, **61**, 313.
551. R.M. Tripathi, S. Mahapatra, R. Raghunath, V.N. Sastry and T.M. Krishnamoorthy, *Sci. Total Environ.*, 2000, **250**, 43.
552. M. Yaman, *Chem. Anal.* (*Warsaw*), 1997, **42**, 79.
553. J. Kalembkiewicz and L. Filar, *Chem. Anal.* (*Warsaw*), 1997, **42**, 87.
554. C. Cabrera-Vique, P.L. Teissedre, M.T. Cabanis and J.C. Cabanis, *Am. J. Enol. Vitic.*, 2000, **51**, 103.
555. S. Bajo, L. Tobler and A. Wyttenbach, *Fresen. J. Anal. Chem.*, 1993, **347**, 344.
556. S. Holzinger, M. Anke, B. Roehrig and D. Gonzalez, *Analyst*, 1998, **123**, 447.
557. E. Sievers, T. Arpe, U. Schleyerbach and J. Schaub, *J. Pediatr. Gastroenterol. Nutr.*, 2000, **31**, 57.
558. S.L.C. Ferreira, W.N.L. dos Santos and V.A. Lemos, *Anal. Chim. Acta*, 2001, **445**, 145.
559. P.L. Teissedre, C.C. Vique, M.T. Cabanis and J.C. Cabanis, *Am. J. Enol. Vitic.*, 1998, 49, 205.
560. M.P.C.V.D. Parmigiani and A.F. Midio, *Analusis*, 1997, **25**, 36.
561. Y.P. de Pena, B. Paredes, W. Rondon, M. Burguera, J.L. Burguera, C. Rondon, P. Carrero and T. Capote, *Talanta*, 2004, **64**, 1351.
562. D. Woolard, T. Franks and D.R. Smith, *J. Anal. At. Spectrom.*, 1998, **13**, 1015.
563. G.M. A Scelfo and R. Flegal, *Environ. Health Perspect.*, 2000, **108**, 309.
564. E.C. Santiago and F.B.B. Bello, *Food Addit. Contam.*, 2003, **20**, 535.
565. M. Kim, C. Kim and I. Song, *Food Addit. Contam.*, 2003, 20, 149.
566. R.G. Wuilloud, H.A. Acevedo, F.A. Vazquez and L.D. Martinez, *Anal. Lett.*, 2002, **35**, 1649.
567. H. Barton, Z. Zachwieja and M. Folta, *Food Addit. Contam.*, 2002, **19**, 906.
568. M.A. Garcia, J. Alonso, M.L. Fernandez and M.J. Melgar, *Arch. Environ. Contam. Toxicol.*, 1998, **34**, 330.
569. M. Chu and D. Beauchemin, *J. Anal. At. Spectrom.*, 2004, **19**, 1213.

570. Z.W. Shen, Y.G. Dong and H.J. Shen, *Spectrosc. Spectr. Anal*., 2000, **20**, 390.
571. J. Marrero, S.P. Arisnabarreta and P. Smichowski, *At. Spectrosc*., 2003, **24**, 133.
572. H.W. Sun, Y. Gao, C.G. Yuan, Y.X. Zhang, L.L. Yang and D.Q. Zhang, *Anal. Sci*., 2002, **18**, 325.
573. S. Cerutti, R.F. Orsi, J.A. Gasquez, R.A. Olsina and L.D. Martinez, *J. Trace Microprobe Tech*., 2003, **21**, 421.
574. J.C.R. Garcia, J.B. Garcia, C.H. Latorre, S.G. Martin and R.M.P. Crecente, *Food Chem*., 2005, **91**, 435.
575. M.A. Saleh, A.A. Ragab, A. Kamel, J. Jones and A. Khalek El-Sebae, *Chemosphere*, 1996, **32**, 1859.
576. C.R. Baum and M.W. Shannon, *J. Toxicol. Clin. Toxicol.,* 1997, **35**, 371.
577. H.H. Ang, E.L. Lee and K. Matsumoto, *Hum. Exp. Toxicol*., 2003, **22**, 445.
578. P. Falomir, A. Alegria, R. Barbera, R. Farre and M.J. Lagarda, *Food Chem*., 1999, **64**, 111.
579. R.C.M. Almeida and S.D.M.T. Vasconcelos, *Anal. Chim. Acta*, 1999, **396**, 45.
580. E.C. Lima, F.J. Krug and M.A.Z. Arruda, *Spectrochim. Acta B*, 1998, **53**, 601.
581. R.W. Dabeka, A.D. McKenzie and K. Pepper, *Food Addit. Contam*., 2002, **19**, 47.
582. Z.W. Zhang, C.S. Moon, T. Watanabe, S. Shimbo and M. Ikeda, *Sci. Total Environ*., 1996, **191**, 169.
583. V.A. Lemos and S.L.C. Ferreira, *Anal. Chim. Acta*, 2001, **441**, 281.
584. P.K. Tewari and A.K. Singh, *Talanta*, 2002, **56**, 735.
585. J.C. Jin and H.W. Chen, *X.Y. Shen*, *Fenxi Huaxue*, 1996, **24**, 957.
586. J.A. Salonia, R.G. Wuilloud, J.A. Gasquez, R.A. Olsina and L.D. Martinez, 1999, *J. Anal. At. Spectrom*., **14**, 1239.
587. O. Acar, Z. Kilic and A.R. Turker, *Food Chem*., 2000, **71**, 117.
588. K. Ndung'u, S. Hibdon and A.R. Flegal, *Talanta*, 2004, **64**, 258.
589. U. Tinggi and G. Craven, *Microchem. J*., 1996, **54**, 168.
590. Y. Xu and Y. Liang, *J. Anal. At. Spectrom*., 1997, **12**, 471.
591. X.Y. Zhang, *Chin. J. Anal. Chem*., 2000, **28**, 1493.
592. S.Y. Bae, X. Zeng and G.M. Murray, *J. Anal. At. Spectrom*., 1998, **13**, 1177.
593. G.H. Tao and Z.L. Fang, *Fresen. J. Anal. Chem*., 1998, **360**, 156.
594. S. Imai, Y. Kubo, A. Yonetani, N. Ogawa and Y. Kikuchi, *J. Anal. At. Spectrom*., 1998, **13**, 1199.
595. Y. Li, Y. Jiang, X.P. Yan, W.J. Peng and Y.Y. Wu, *Anal. Chem*., 2002, **74**, 1075.
596. C.M.R. Almeida and M.T.S.D. Vasconcelos, *J. Anal. At. Spectrom*., 1999, **14**, 1815.
597. X.D. Tian, H. Emteborg, M. Barbaste and F.C. Adams, *J. Anal. At. Spectrom*., 2000, **15**, 829.
598. R.G. Wuilloud, A.H. Gonzalez, E.J. Marchevsky, R.A. Olsina and L.D. Martinez*, J. AOAC Int*., 2001, **84**, 1555.
599. A. Kocsonya, O. Guguianu, I. Demeter, K. Hollos-Nagy, I. Kovacs and Z. Szokefalvi-Nagy, *Nucl. Instrum. Methods Phys. Res. B*, 2002, **189**, 511.
600. C.R. Quetel, S.M. Nelms, L. Van Nevel, I. Papadakis and P.D.P. Taylor, *J. Anal. At. Spectrom*., 2001, **16**, 1091.
601. S. Augagneur, B. Medina and F.E. Grousset, *Fresen. J. Anal. Chem*., 1997, **357**, 1149.

602. C.S. Stockley, L.H. Smith, K.G. Tiller, B.L. Gulson, C.D. Osborn and T.H. Lee, *Aust. J. Grape Wine Res*., 2003, **9**, 47.
603. R. Larcher, G. Nicolini and P. Pangrazzi, *J. Agric. Food Chem*., 2003, **51**, 5956.
604. B. Medina, S. Augagneur, M. Barbaste, F.E. Grouset and P. Buat-Meard, *Food Addit. Contam*., 2000, **17**, 435.
605. C.M. Mena, C. Cabrera, M.L. Lorenzo and M.C. Lopez, *J. Agric. Food Chem*., 1997, **45**, 1812.
606. V.I. Slaveykova, D.L. Tsalev and R. Maskill, *Ciencia* (*Maracaibo*),1996, **4**, 165.
607. J. Szpunar, P. Pellerin, A. Makarov, T. Doco, P. Williams, B. Medina and R. Lobinski, *J. Anal. At. Spectrom.*, 1998, **13**, 749.
608. S.C. Hight, *Food Addit. Contam*., 1996, **13**, 747.
609. A. Kaufmann, *Food Addit. Contam*., 1998, **15**, 437.
610. V.A. Lemos, M. de la Guardia and S.L.C. Ferreira, *Talanta*, 2002, **58**, 475.
611. Z.Y. Zuo, M. Zhang, Z.A. Sun and D.S. Wang, *Spectrosc. Spectr. Anal*., 2002, **22**, 859.
612. P.A. Brereton, P. Robb, C.M. Sargent, H.M. Crews and R. Wood, *J. AOAC Int*., 1997, **80**, 1287.
613. C.M.R. Almeida and M.T.S.D. Vasconcelos, *J. Agric. Food Chem*., 2003, **51**, 3012.
614. M. Barbaste, L. Halicz, A. Galy, B. Medina, H. Emteborg, F.C. Adams and R. Lobinski, *Talanta*, 2001, **54**, 307.
615. T. Perez-Corona, Y. Madrid and C. Camara, *Anal. Chim. Acta*, 1997, **345**, 249.
616. M. Montes-Bayon, D.L. LeDuc, N. Terry and J.A. Caruso, *J. Anal. At. Spectrom*., 2002, **17**, 872.
617. A.P. Vonderheide, K. Wrobel, S.S. Kannamkumarath, C. B'Hymer, M. Montes-Bayon, C.P. De Leon and J.A. Caruso, *J. Agric. Food Chem*., 2002, **50**, 5722.
618. M.T. Roberge, A.J. Borgerding and J.W. Finley*, J. Agric. Food Chem*., 2003, **51**, 4191.
619. M. Stadlober, M. Sager and K.J. Irgolic, *Food Chem*., 2001, **73**, 357.
620. P.C. Aleixo, J.A. Nobrega, D. Santos and P.C.S. Muller, *Quim. Nova*, 2000, **23**, 310.
621. V.D. Huerta, M.L.F. Sanchez and A. Sanz-Medel, *J. Anal. At. Spectrom*., 2004, **19**, 644.
622. H.M. Crews, P.A. Clarke, D.J. Lewis, L.M. Owen, P.R. Strutt and A.J. Izquierdo, *J. Anal. At. Spectrom*., 1996, **11**, 1177.
623. J. Meija, J.M. Bryson, A.P. Vonderheide, M. Montes-Bayon and J.A. Caruso, *J. Agric. Food Chem.,* 2003, **51**, 5116.
624. Y. Tamari, *Bunseki Kagaku*, 2000, **49**, 381.
625. M. Korenovska, *Chem. Pap.-Chem. Zvesti*, 2003, **57**, 155.
626. K.L. Sutton, C.A. Ponce de Leon, K.L. Ackley, R.M.C. Sutton, A.M. Stalcup and J.A. Caruso, *Analyst*, 2000, **125**, 281.
627. L. Valiente, M. Piccinna, E.R. Ale, A. Grillo and P. Smichowski, *At. Spectrosc*., 2002, **23**, 129.
628. J. Zheng, H. Greschonig, F. Liu and W. Kosmus, *Trace Elem. Electrolyt*., 2000, **17**, 40.

629. A. Lopez-Molinero, R. Gimenez, P. Otal, A. Callizo, P. Charmorro and J.R. Castillo, *J. Anal. At. Spectrom*., 2002, **17**, 352.
630. M. Guidotti, *J. AOAC Int*., 2000, **83**, 1082.
631. G. Onning and I.A. Bergdahl, *Analyst*, 1999, **124**, 1435.
632. G.S.B. Januzzi, F.J. Krug, and A.Z. Arruda, *J. Anal. At. Spectrom*., 1997, **12**, 375.
633. V. Satovic, D. Beker and L. Gumhalter-Karolyi, *Eur. Food Res. Technol*., 2003, **217**, 154.
634. P. Moreno, M.A. Quijano, A.M. Gutierrez, M.C. Perez-Conde and C. Camara, *Anal. Chim. Acta*, 2004, **524**, 315.
635. J.P. Diaz, M. Navarro, H. Lopez and M.C. Lopez, *Food Addit. Contam*., 1997, **14**, 109.
636. P. Smrkolj, L. Pograjc, C. Hlastan-Ribic and V. Stibilj, *Food Chem*., 2005, **90**, 691.
637. L. Gamiz-Gracia and M.D. Luque de Castro, *Talanta*, 1999, **50**, 875.
638. F.M. Al-Awadi and T.S. Srikumar, *J. Trace Elem. Exp. Med*., 2001, **14**, 57.
639. J. Murphy and K.D. Cashman, *Food Chem*., 2001, **74(4)**, 493.
640. V.M.C. Dias, S. Cadore and N. Baccan, *J. Anal. At. Spectrom*., 2003, **18**, 783.
641. J. Kumpulainen and J.E. Saarela, *J. Anal. At. Spectrom*., 1992, **7**, 165.
642. T. Klapec, M.L. Mandic, J. Grgic, L. Primorac, A. Perl and V. Krstanovic, *Food Chem*., 2004, **85**, 445.
643. T. Narukawa, *J. Anal. At. Spectrom*., 1999, **14**, 1919.
644. Y.M. Liu, B.L. Gong, Z.H. Li, Y. Xu and T.H. Lin, *Talanta*, 1996, **43**, 985.
645. J.L. Burguera, P. Carrero, M. Burguera, C. Rondon, M.R. Brunetto and M. Gallignani, *Spectrochim. Acta B*, 1996, **51B**, 1837.
646. E. Puskel, Z. Mester and P. Fodor, *J. Anal. At. Spectrom*., 1999, **14**, 973.
647. Y. Ogra and K.T. Suzuki, *J. Anal. At. Spectrom*., 2005, **20**, 35.
648. M. Kotrebai, M. Birringer, J.F. Tyson, E. Block and P.C. Uden, *Anal. Commun.,* 1999, **36**, 249.
649. C. Ip, M. Birringer, E. Block, M. Kotrebai, J.F. Tyson, P.C. Uden and D.J. Lisk, *J. Agric. Food Chem*., 2000, **48**, 2062.
650. M. Shah, S.S. Kannamkumarath, J.C.A. Wuilloud, R.G. Wuilloud and J.A. Caruso, *J. Anal. At. Spectrom.*, 2004, **19**, 381.
651. F.M. Al-Awadi and T.S. Srikumar, *J. Trace Elem. Exp. Med*., 2001, **14**, 57.
652. A. Alegria, R. Barbera, R. Farre, E. Ferrer, M.J. Lagarda and M.A. Torres, *J. AOAC Int*., 1998, **81**, 457.
653. E.-S. Kim, H.-K. Keum and Y. Tamari, *J. Food Sci. Nutr*., 1996, **1**, 230.
654. K.K. Cook, *J. AOAC Int*., 1996, **79**, 1162.
655. A.E. El-Arab and J. Bruggemann, *Int. J. Food Sci. Nutr*., 2003, **54**, 175.
656. U. Tinggi, *J. AOAC Int*., 1999, **82**, 364.
657. P. Cava-Montesinos, M.L. Cervera, A. Pastor and M. de la Guardia, *Anal. Chim. Acta*, 2003, **481**, 291.
658. L.H. Foster and S. Sumar, *Food Chem*., 1995, **53**, 453.
659. X.M. Guo and X.W. Guo, *J. Anal. At. Spectrom*., 2001, **16**, 1414.
660. Z. Stefanka, I. Ipolyi, M. Dernovics and P. Fodor, *Talanta*, 2001, **55**, 437.
661. L. Racz, A. Bumbalova, M. Harangozo, J. Tolgyessy and O. Tomecek, *J. Radioanal. Nucl. Chem*., 2000, **246**, 611.
662. C. Brunori, M.B. DelaCalle, M. Angelone and R. Morabito, *Ann. Chim*., 1999, **89**, 873.

663. D.Q. Zhang, H.W. Sun and L.L. Yang, *Fresen. J. Anal. Chem*., 1997, **359**, 492.
664. M.V. Pelaez, M.M. Bayon, J.I.G. Alonso and A. Sanz-Medel, *J. Anal. At. Spectrom*., 2000, **15**, 1217.
665. S.S. Kannamkumarath, K. Wrobel, A. Vonderheide and J.A. Caruso, *Anal. Bioanal. Chem*., 2002, **373**, 454.
666. K. Wrobel, K. Wrobel, S.S. Kannamkumarath, J.A. Caruso, I.A. Wysocka, E. Bulska, J. Swiatek and M. Wierzbicka, *Food Chem*., 2004, **86**, 617.
667. A. Amokrane and M.E.A. Benamar, *Nucl. Instrum. Methods Phys. Res. A*, 2002, **480**, 50.
668. P. Moreno, M.A. Quijano, A.M. Gutierrez, M.C. Perez-Conde and C.R. Camara, *J. Anal. At. Spectrom*., 2001, **16**, 1044.
669. P. Moreno, M.A. Quijano, A.M. Gutierrez, M.C. Perez-Conde and C. Camara, *Anal. Bioanal. Chem.*, 2002, **374**, 466.
670. S. Boulyga, J. Dombovari, J.S. Becker and H.J. Dietze, *At. Spectrosc*., 2000, **21**, 149.
671. C. Dietz, J.S. Landaluze, P. Ximenez-Embun, Y. Madrid-Albarran and C. Camara, *Anal. Chim. Acta*, 2004, **501**, 157.
672. L.C. Chen, F.M. Yang, J. Xu, Y. Hu, Q.H. Hu, Y.L. Zhang and G.X. Pan, *J. Agric. Food Chem*., 2002, **50**, 5128.
673. Q.H. Hu, L.C. Chen, J. Xu, Y.L. Zhang and G.X. Pan, *J. Sci. Food Agric*., 2002, **82**, 869.
674. J.L. Gomez-Ariza, M.A.C. de la Torre, I. Giraldez, D. Sanchez-Rodas, A. Velasco and E. Morales, *Appl. Organomet. Chem*., 2002, **16**, 265.
675. H. Mendez, F. Alava, I. Lavilla and C. Bendicho, *Anal. Chim. Acta*, 2002, **452**, 217.
676. L.C. Chien, C.Y. Yeh, S.Y. Huang, M.J. Shieh and B.C. Han, *Sci. Total Environ*., 2003, **311**, 57.
677. C.A. Ponce de Leon, K.L. Sutton, J.A. Caruso and P.C. Uden, *J. Anal. At. Spectrom*., 2000, **15**, 1103.
678. B. Michalke and P. Schramel, *J. Chromatogr. A*, 1998, **807**, 71.
679. P.C. Uden, H.T. Boakye, C. Kahakachchi, R. Hafezi, P. Nolibos, E. Block, S. Johnson, and J.F. Tyson, *J. Anal. At. Spectrom*., 2004, **19**, 65.
680. L. Bendahl and B. Gammelgaard, *J. Anal. At. Spectrom*., 2004, **19**, 143.
681. H.G. Infante, G. O'Connor, M. Rayman, R. Wahlen, J. Entwisle, P. Norris, R. Hearn, T. Catterick, *J. Anal. At. Spectrom*., 2004, **19**, 1128.
682. L.H. Foster and S. Sumar, *Food Chem*., 1996, **56**, 93.
683. F.M. Yang, L.C. Chen, Q.H. Hu and G.X. Pan, *Biol. Trace Elem. Res*., 2003, **93**, 249.
684. Y. Tan and W.D. Marshall, *Analyst*, 1997, **122**, 13.
685. H. Ge, X.J. Cai, J.F. Tyson, P.C. Uden, E.R. Denoyer and E. Block, *Anal. Commun*., 1996, **33**, 279.
686. P. Smrkolj and V. Stibilj, *Anal. Chim. Acta*, 2004, **512**, 11.
687. J.M. Gonzalez Lafuente, M.L. Fernandez Sanchez and A. Sanz-Medel, *J. Anal. At. Spectrom*., 1996, **11**, 1163.
688. M.B. de La Calle-Guntinas, C. Brunori, R. Scerbo, S. Chiavarini, P. Quevauviller, F. Adams and R. Morabito, *J. Anal. At. Spectrom*., 1997, **12**, 1041.

689. M. Langenauer and U. Kraehenbuhl, *Chimia*, 1991, **45**, 8.
690. H. Emteborg, G. Bordin and A.R. Rodriguez, *Analyst*, 1998, **123**, 245.
691. J. Jaganathan and S.M. Dugar, *Am. J. Enol. Vitic*., 1998, **49**, 115.
692. J. Cvetkovic, T. Stafilov and D. Mihajlovic, *Fresen. J. Anal. Chem*., 2001, **370**, 1077.
693. S. Perez Mendez, E. Blanco Gonzalez and A. Sanz Medel, *J. Anal. At. Spectrom*., 2000, **15**, 1109.
694. C. Casiot, V. Vacchina, H. Chassaigne, J. Szpunar, M. Potin-Gautier and R. Lobinski, *Anal. Commun*., 1999, **36**, 77.
695. C. Casiot, J. Szpunar, R. Lobinski and M. Potin-Gautier, *J. Anal. At. Spectrom*., 1999, **14**, 645.
696. S.M. Bird, P.C. Uden, J.F. Tyson, E. Block and E. Denoyer, *J. Anal. At. Spectrom*., 1997, **12**, 785.
697. K. Wrobel and J.A. Caruso, *J. Anal. At. Spectrom*., 2002, **17**, 1048.
698. N.E. Renard and T.A. Tompkins, *Biol. Trace Elem. Res*., 2002, **88**, 185.
699. T. Lindemann and H. Hintelmann, *Anal. Chem*., 2002, **74**, 4602.
700. H. Chassaigne, C.C. Chery, G. Bordin and A.R. Rodriguez, *J. Chromatogr. A*, 2002, **976**, 409.
701. J.R. Encinar, L. Ouerdane, W. Buchmann, J. Tortajada, R. Lobinski and J. Szpunar, *Anal. Chem*., 2003, **75**, 3765.
702. J.L. Gomez-Ariza, M.A.C. de la Torre, I. Giraldez and E. Morales, *Anal. Chim. Acta*, 2004, **524**, 305.
703. E.H. Larwn and J. Ekelund, *Analyst*, 1989, **114**, 915.
704. P. Braga, M. Montes-Bayon, J. Alvarez, J.M. Lopez and A. Sanz-Medel, *J. Anal. At. Spectrom*., 2004, **19**, 1529.
705. A. Polatajko, M. Sliwka-Kaszynska, M. Dernovics, R. Ruzik, J.R. Encinar and J. Szpunar, *J. Anal. At. Spectrom*., 2004, **19**, 114.
706. E. Dumont, K. De Cremer, M. Van Hulle, C.C. Chery, F. Vanhaecke and R. Cornelis, *J. Anal. At. Spectrom*., 2004, **19**, 167.
707. L. Yang, R.E. Sturgeon, W.R. Wolf, R.J. Goldschmidt and Z. Mester, *J. Anal. At. Spectrom*., 2004, **19**, 1448.
708. C. Dietz, J.S. Landaluze, P. Ximenez-Embun, Y. Madrid-Albarran and C. Camara, *J. Anal. At. Spectrom*., 2004, **19**, 260.
709. C.C. Chery, H. Chassaigne, L. Verbeeck, R. Cornelius, F. Vanhaecke, L. Moens, *J. Anal. At. Spectrom*., 2002, **17**, 576.
710. S. McSheehy, F. Pannier, J. Szpunar, M. Potin-Gautier and R. Lobinski, *Analyst*, 2002, **127**, 223.
711. C. B′Hymer and J.A. Caruso, *J. Anal. At. Spectrom*., 2000, **15**, 1531.
712. R. Rezaaiyan and S.J. Nikdel, *J. Food Sci*., 1990, **55**, 1350.
713. K.K. Van Dyck, H. Robberecht, R. Van Cauwenbergh, H. Deelstra, J. Arnaud, L. Willemyns, F. Benijts, J.A. Centeno, H. Taylor, M.E. Soares, M.L. Bastos, M.A. Ferreira, P.C. D'Haese, L.V. Lamberts, M. Hoenig, G. Knapp, S.J. Lugowski, L. Moens, J. Riondato, R. Van Grieken, M. Claes, R. Verheyen, L. Clement and M. Uytterhoeven, *J. Anal. At. Spectrom*., 2000, **15(6)**, 735.
714. W. Dejneka and J. Lukasiak, *Food Control*, 2003, **14**, 193.

715. K. Van Dyck, H. Robberecht, R. Van Cauwenbergh and H. Deelstra, *Eur. Food Res. Technol*., 1999, **210**, 77.
716. S.J. Lugowski, D.C. Smith, H. Bonek, J. Lugowski, W. Peters and J. Semple, *J. Trace Elem. Med. Biol*., 2000, **14**, 31.
717. P. Bermejo-Barrera, M.C. Barciela-Alonso, R. Dominguez-Gonzalez, A. Bermejo-Barrera, J.A.C. de Juan and J.M. Fraga-Bermudez, *Anal. Bioanal. Chem*., 2002, **374**, 1290.
718. K. Matsusaki, T. Yamaguchi and T. Sata, *Anal. Sci*., 1996, **12**, 797.
719. X.S. Wang, Z.C. Long, L.M. Song, G.Z. Ma and J.Z. Wei, Chin. *J. Anal. Chem*., 2002, **30**, 1214.
720. L. Perring and M. Basic-Dvorzak, *Anal. Bioanal. Chem*., 2002, **374**, 235.
721. S.J. Kumar, M.A. Reddy and N.N. Meeravali, *J. Anal. At. Spectrom*., 2005, **20**, 124.
722. Z. Qunfang, J. Guibin, L. Zhongyang, L. Lina, Y. Chungang and W. Yongning, *Food Addit. Contam*., 2004, **21**, 1162.
723. G.H. Biego, H. Biaudet, M. Joyeux and G. Debry, *Sci. Aliments*, 1997, **16**, 623.
724. W.A.C. Anderson, S.A. Thorpe, L.M. Owen, S.E. Anderson, H.M. Crews and S.L. Reynolds, *Food Addit. Contam.,* 1998, **15**, 288.
725. K. Ide, K. Sato and S. Hashimoto, *Bunseki Kagaku*, 2000, **49**, 795.
726. V.O. Schmitt, J. Szpunar, O.F.X. Donard and R. Lobinski, *Can. J. Anal. Sci. Spectrosc*., 1997, **42**, 41.
727. G.C. Sturniolo, C. Mestriner, P. Irato, V. Albergoni, G. Longo and R. D'Inca, *Am. J. Gastroenterol*., 1999, **94**, 334.
728. P. Bermejo-Barrera, T. Pardinas-Alvite, M.C. Barciela-Alonso, A. Bermejo-Barrera, J.A. Cocho de Juan and J.M. Fraga-Bermudez, *J. Anal. At. Spectrom*., 2000, **15**, 435.
729. M.Q. Zou, T.S. Jiang, D.N. Wang and Q.Z. Zai, *Rare Metals*, 2003, **22**, 14.
730. P.L. Teissedre, M. Krosniak, K. Portet, F. Gasc, A.L. Waterhouse, J.J. Serrano, J.C. Cabanis and G. Cros, *Food Addit. Contam*., 1998, **15**, 585.
731. G. Schwedt, A.B. Tawali and K. Koch, *Fresen. J. Anal. Chem*., 1998, **360**, 589.
732. M. Schlegel-Zawadzka, Z. Zachwieja, A. Huzior-Baajewicz and J.J. Pietrzyk, *Food Addit. Contam*., 2002, **19**, 963.
733. H.W. Sun, R. Suo and Y.K. Lu, *Anal. Chim. Acta*, 2002, **457**, 305.
734. L. Hussein and J. Bruggeman, *Food Chem*., 1997, **58**, 391.
735. I. Lopez-Garcia, P. Vinas, C. Blanco and M. Hernandez-Cordoba, *Talanta*, 1999, **49**, 597.
736. J. Arnaud, A. Favier and J. Alary, *J. Anal. At. Spectrom*., 1991, **6**, 647.
737. D. Bosscher, Z.L. Lu, G. Janssens, M. Van Caillie-Bertrand, H. Robberecht, H. De Rycke, R. De Wilde and H. Deelstra, *Br. J. Nutr*., 2001, **86**, 241.
738. A. Krushevska, R.M. Barnes, C.J. Amarasiriwaradena, H. Foner and L. Martines, *J. Anal. At. Spectrom.*, 1992, **7**, 851.
739. P. Bermejo, R. Dominguez, A. Reboiro, A. Bermejo, J.M. Fraga and J.A. Cocho de Juan, *At. Spectrosc*., 1999, **20**, 161.
740. M.A. de la Fuente, F. Montes, G. Guerrero and M. Juarez, *Food Chem*., 2003, **80**, 573.

741. F. Corradini, L. Marcheselli, A. Marchetti, C. Petri and C. Biancardi, *J. AOAC Int*., 1994, **77**, 714.
742. D. Pozebon, V.L. Dressler and A.J. Curtius, *J. Anal. At. Spectrom*., 1998, **13**, 1101.
743. A. Hardisson, C. Rubio, A. Baez, M.M. Martin and R. Alvarez, *Eur. Food Res. Technol.,* 2001, **213**, 225.
744. L. Jorhem, B. Sundstrom, J. Engman, C. Astrand-Yates and I. Olsson, *Food Addit. Contam*., 1996, **13**, 737.
745. H. Matusiewicz and M. Kopras, *J. Anal. At. Spectrom*., 1997, **12**, 1287.
746. P. Pohl and B. Prusisz, *Anal. Chim. Acta*, 2004, **502**, 83.
747. H. Matusiewicz and M. Mikolajczak, *J. Anal. At. Spectrom*., 2001, **16**, 652.
748. F. Ronda, D. Sancho, M. del Alamo and M. Gomez, *Zuckerindustrie*, 2001, **126**, 208.
749. I. Matsushige and E. de Oliveira, *Food Chem.*, 1993, **47**, 205.
750. B. Demirözü, I. Saldamlı, B. Gürsel, A. Uçak, F. Cetinyokus, and N. Yuzbasi, *J. Cereal Sci*., 2003, **37**, 171.
751. V.A. Maihara and M.B.A. Vasconcellos, *J. Radioanal. Nucl. Chem. Ar*., 1988, **122**, 161.
752. B. Bocca, A. Alimonti, E. Coni, M. Di Pasquale, L. Giglio, A.P. Bocca and S. Caroli, *Talanta,* 2000, **53**, 295.
753. R. Forrer, K. Gautschi, A. Stroh and H. Lutz, *J. Trace Elem. Med. Biol*., 1999, **12**, 240.
754. F.M. Al-Awadi and T.S. Srikumar, *J. Dairy Res*., 2001, **68**, 463.
755. L.J. Melnyk, J.N. Morgan, R. Fernando, E.D. Pellizzari and O. Akinbo, *J. AOAC Int*., 2003, **86**, 439.
756. A.A. Al-Warthan and H.M. Al-Swaidan, *Arab Gulf J. Sci. Res*., 1995, **13**, 453.
757. D.R. Fernandez, D.J. Vanderjagt, M. Williams, Y.-S. Huang, L.-T. Chuang, M. Millson, R. Andrews, A. Pastuszyn and R.H. Glew, *Plant Foods Hum. Nutr*., 2002, **57**, 257.
758. X.D. Hou, H.L. Peters, Z. Yang, K.A. Wagner, J.D. Batchelor, M.M. Daniel and B.T. Jones, *Appl. Spectrosc*., 2003, **57**, 338.
759. S.I. Segura-Munoz, T.M.B. Trevilato, A.M.M. Takayanagui, S.E. Hering and P. Cupo, *Arch. Latinoam. Nutr*., 2003, **53**, 59.
760. F. Cubadda, A. Raggi, F. Zanasi and M. Carcea, *Food Addit. Contam*., 2003, **20**, 353.
761. P. Ruperez, *Food Chem*., 2002, **79**, 23.
762. H.H. Cho, Y.J. Kim, Y.S. Jo, K. Kitagawa, N. Arai and Y.I. Lee, *J. Anal. At. Spectrom*., 2001, **16**, 622.
763. R. Klockenkaemper and A. von Bohlen, *X-Ray Spectrom*., 1996, **25**, 156.
764. H.M. Crews, *Spectrochim. Acta B*, 1998, **53**, 213.
765. D.L. Anderson, R.G. Downing and G.V. Iyengar, *Fresen. J. Anal. Chem*., 1995, **352**, 107.
766. P. Jacob and H.J. Berndt, *J. Anal. At. Spectrom.*, 2002, **17**, 1615.
767. M.K. Turkdogan, F. Kilicel, K. Kara, I. Tuncer and I. Uygan, *Environ. Toxicol. Pharmacol*., 2003, **13**, 175.
768. P. Zbinden and D. Andrey, *At. Spectrosc*., 1998, **19**, 214.
769. H.M. Crews, *Trace Element Analysis of Food Authenticity Studies*. *Analytical Methods Food Authentication*, Blackie, London, UK, 1998, 270–291.

770. M. Careri, F. Bianchi and C. Corradini, *J. Chromatogr. A*, 2002, **970**, 3.
771. R. Dybczynski, *Food Addit. Contam*., 2002, **19**, 928.
772. L. Jorhem, *J. AOAC Int*., 1993, **76**, 798.
773. H. Muto, T. Abe, Y. Takizawa, K. Kawabata, K. Yamaguchi and K. Saitoh, *Sci. Total Environ*. 1994, **144**, 231.
774. R. Barbera, R. Farre and D. Mesado, *Nahrung-Food*, 1991, **35**, 683.
775. R.L. Sims, R.M. Mullen and D.B. Milne, *J. Food Compos. Anal*., 1990, **3**, 27.
776. S.J. Parry and D.S.J. Aston, *Food Addit. Contam*., 2004, **21**, 506.
777. J.M. Llobet, S. Granero, M. Schuhmacher, J. Corbella and J.L. Domingo, *Trace Elem. Electrolytes*, 1998, **15**, 136.
778. I. Roelandts, *Spectrochim. Acta B*, 1997, **52B**, 1073.
779. E. Garcia, C. Cabrera, M.L. Lorenzo, J. Sanchez and M.C. Lopez, *Br. J. Nutr*., 2001, **86**, 391.
780. T. Roychowdhury, H. Tokunaga and M. Ando, *Sci. Total Environ*., 2003, **308**, 15.
781. Z. Kilic, O. Acar, M. Ulasan and M. Ilim, *Food Chem.*, 2002, **76**, 107.
782. V. Orescanin, A. Katunar, A. Kutle and V. Valkovic, *J. Trace Microprobe Tech*., 2003, **21**, 171.
783. M.S. Dundar, E. Bahcivanci and C. Muslu, *Acta Chim. Slov*., 2002, **49**, 537.
784. B. Jamoussi, M. Zafaouf and B. Ben Hassine, *Int. J. Environ. Anal. Chem*., 1996, **61**, 249.
785. R. Fernandez-Torres, J.L. Perez-Bernal, M.A. Bello-Lopez, M. Callejon-Mochon, J.C. Jimenez-Sanchez and A. Guiraum-Perez, *Talanta*, 2005, **65**, 686.
786. M. Yaman and N. Cokol, *At. Spectrosc*., 2004, **25**, 185.
787. F.A.R. Martino, M.L.F. Sanchez and A. Sanz-Medel, *J. Anal. At. Spectrom*., 2002, **17**, 1271.
788. R.W. Dabeka and A.D. McKenzie, *J. AOAC Int*., 1992, **75**, 954.
789. K.K. Cook, *J. AOAC Int*., 1997, **80**, 834.
790. N. Yuzbasi, E. Sezgin, M. Yildirim and Z. Yildirim, *Food Addit. Contam*., 2003, **20**, 464.
791. C. Cabrera, F. Lloris, R. Gimenez, M. Olalla and M.C. Lopez, *Sci. Total Environ*., 2003, **308**, 1.
792. J.A. Brisbin and J.A. Caruso, *Analyst*, 2002, **127**, 921.
793. R.M. Garcia-Rey, R. Quiles-Zafra and M.D.L. de Castro, *Anal. Bioanal. Chem*., 2003, **377**, 316.
794. G.H. Biego, M. Joyeux, P. Hartemann and G. Debry, *Food Addit. Contam*., 1998, **15**, 775.
795. D. Wagley, G. Schmiedel, E. Mainka and H.J. Ache, *At. Spectrosc*., 1989, **10**, 106.
796. P. Kump, M. Necemer and M. Veber, *X-Ray Spectrom*., 1997, **26**, 232.
797. K. Zimmermannova, L. Svoboda and P. Kalac, *Ekol. Bratisl*., 2001, **20**, 440.
798. I. Karadjova, G. Zachariadis, G. Boskou and J. Stratis, *J. Anal. At. Spectrom*., 1998, **13**, 201.
799. M.S. Jimenez, R. Velarte, M.T. Gomez and J.R. Castillo, *At. Spectrosc*., 2004, **25**, 1.
800. J. Alvarez, L.M. Marco, J. Arroyo, E.D. Greaves and R. Rivas, *Spectrochim. Acta B*, 2003, **58**, 2183.
801. L. Castle, C.P. Offen, M.J. Baxter and J. Gilbert, *Food Addit. Contam*., 1997, **14**, 35.

802. L. Favretto, M.G. Pertoldi, L. Gabriel Favretto and D. Vojnovic, *Anal. Chim. Acta*, 1987, **201**, 253.
803. D. Hammer, M. Basic-Drvorzak and L. Perring, *At. Spectrosc*., 2004, **25**, 30.
804. V. Nischwitz, B. Michalke and A. Kettrup, *J. Anal. At. Spectrom*., 2003, **18**, 444.
805. M. Feuerstein and G. Schlemmer, *At. Spectrosc*., 1999, **20**, 149.
806. V. Rewatkar, A.A. Saoji and K.G. Rewatkar, *Asian J. Chem*., 2001, **13**, 206.
807. I. Al-Saleh and N. Shinwari, *Biol. Trace Elem. Res*., 2001, **83**, 91.
808. V.A. Maihara and M.B.A. Vasconcellos, *J. Radioanal. Nucl. Chem*., 1989, **132**, 329.
809. C.J. Park, J.K. Suh, K.W. Lee and S.H. Lee, *Anal. Sci*., 1997, **13**(Suppl. IV), 429.
810. C.R.T. Tarley, W.K.T. Coltro, M. Matsushita and N.E. de Souza, *J. Food Compos. Anal*., 2001, **14**, 611 .
811. P. Bermejo-Barrera, A. Moreda-Pineiro and A. Bermejo-Barrera, *Talanta*, 2002, **57**, 969.
812. E.H. Larsen, G.A. Pedersen and J.W. McLaren, *J. Anal. At. Spectrom*., 1997, **12**, 963.
813. W. A Grant and P.C. Ellis, *J. Anal. At. Spectrom*., 1988, **3**, 815.
814. K. Takiyama and Y. Ishil, *Anal. Sci*., 1992, **8**, 410.
815. M. Lopez-Artiguez, A.M. Camean and M. Repetto, *J. AOAC Int*., 1996, **79**, 1191.
816. F.A. Chmilenko and L.V. Baklanova, *J. Anal. Chem*. (*Transl. of Zh. Anal. Khim.*), 1997, **52**, 1093.
817. E. Zeiller, M.J. Campbell and R. Dekner, *J. Anal. At. Spectrom*., 1997, **12**, 1011.
818. L.B. Allen, P.H. Siitonen and H.C. Thompson Jr., *J. Agric. Food Chem*., 1997, **45**, 162.
819. M.A. Herrador and A.G. Gonzalez, *Talanta*, 2001, **53**, 1249.
820. Y. Ozdemir and S. Gucer, *Chim. Acta Turc*., 1997, **25**, 89.
821. K.E. Odegard and W. Lund, *J. Anal. At. Spectrom*., 1997, **12**, 403.
822. M.J. Salvador, G.N. Lopes, V.F. Nascimento and O.L.A.D. Zucchi, *X-Ray Spectrom*., 2002, **31**, 141.
823. M. Martin-Polvillo, T. Albi and A. Guinda, *J. Am. Oil Chem. Soc*., 1994, **71**, 347.
824. R. Calapaj, S. Chiricosta, G. Saija and E. Bruno, *At. Spectrosc*., 1988, **9**, 107.
825. E. Wieteska, A. Zioek and A. Drzewinska, *Anal. Chim. Acta*, 1996, **330**, 251.
826. C.A.P. de Leon, M. Montes Bayon and J.A. Caruso, *Anal. Bioanal. Chem*., 2002, **374**, 230.
827. V. Stefanova, V. Kmetov, L. Futekov and D. Georgieva, *Anal. Lab*., 1997, **6**, 31.
828. A. Maquieira, H.A.M. Elmahadi and R. Puchades, *Analyst* 1996, **121**, 1633.
829. Y.T. Li, J.C. Van Loon and R.R. Barefoot, *Fresen. J. Anal. Chem*., 1993, **345**, 467.
830. L. Jorhem, B. Sundstrom and J.N. Engman, *J. AOAC Int*., 2001, **84**, 1984.
831. B. Demirozu-Erdinc and I. Saldamli, *Int. J. Dairy Technol*., 2000, **53**, 96.
832. J. Kristl, M. Veber and M. Slekovec, *Acta Chim. Slov*., 2003, **50**, 123.

833. M.E.G. Huguet, *At. Spectrosc*., 2004, **25**, 177.
834. M.I. Guerrero, C. Herce-Pagliai, A.M. Camean, A.M. Troncoso and A. Gustavo Gonzalez, *Talanta*, 1997, **45**, 379.
835. J.S. Becker, M. Zoriy, U. Krause-Buchholz, J.S. Becker, C. Pickhardt, M. Przybylski, W. Pompe and G. Rodel, *J. Anal. At. Spectrom*., 2004, **19**, 1236.
836. Z.F. Chai, Z.Y. Zhang, W.Y. Feng, C.Y. Chen, D.D. Xu and X.L. Hou, *J. Anal. At. Spectrom*., 2004, **19**, 26.
837. F.O. Nwosu, F.A. Adekola and E. Ibitoye, *Bull. Chem. Soc. Ethiop*., 2003, **17**, 1.
838. B.L. Gulson, K.J. Mizon, M.J. Korsch, K.R. Mahaffey and A.J. Taylor, *Environ. Res*., 2001, **87**, 160.
839. J.M. Marchante-Gayon, C.S. Muniz, J.I.G. Alonso and A. Sanz-Medel, *Anal. Chim. Acta*, 1999, **400**, 307.
840. H. Matsuura, A. Hokura, F. Katsuki, A. Itoh and H. Haraguchi, *Anal. Sci*., 2001, **17**, 391.
841. J.R.W. Woittiez, G.V. Iyengar and Z. Fresenius, *Anal. Chem*., 1988, **332**, 657.
842. M.J. Martin, F. Pablos and A.G. Gonzalez, *Anal. Chim. Acta*, 1998, **358**, 177.
843. A.S. Ribeiro, A.L. Moretto, M.A.Z. Arruda and S. Cadore, *Microchim. Acta*, 2003, **141**, 149.
844. P. Bermejo-Barrera, A. Moreda-Pineiro, O. Muniz-Naveiro, A.M.J. Gomez-Fernandez and A. Bermejo-Barrera, *Spectrochim. Acta B*, 2000, **55**, 1351.
845. M.S. Jimenez, R. Velarte and J.R. Castillo, *J. Anal. At. Spectrom*., 2003, **18**, 1154.
846. S. Frias, J.E. Conde, M.A. Rodriguez, V. Dohnal and J.P. Perez-Trujillo, *Nahrung-Food*, 2002, **46**, 370.
847. C. Skinner, N.J. Turoczy, P.L. Jones, D. Barnett and R. Hodges, *Food Chem*., 2004, **85**, 351.
848. A. Wagner and J. Boman, *Spectrochim. Acta B*, 2003, **58**, 2215.
849. S. Caroli, G. Forte, A.L. Iamiceli and B. Galoppi, *Talanta*, 1999, **50**, 327.
850. T. Alkanani, J.K. Friel, S.E. Jackson and H.P. Longerich, *J. Agric. Food Chem*., 1994, **42**, 1965.
851. M. Gonzalez, L. Tapia, M. Alvarado, J.D. Tornero and R. Fernandez, *J. Anal. At. Spectrom*., 1999, **14**, 885.
852. F.L. Cheng, M.X. Ning, J.Y. Mo and Y.C. Liang, *Spectrosc. Spectr. Anal*., 2002, **22**, 676.
853. P. Bermejo-Barrera, O. Muniz-Naveiro, A. Moreda-Pineiro and A. Bermejo-Barrera, *Anal. Chim. Acta*, 2001, **439**, 211.
854. C.F. Wang, C.H. Ke and J.Y. Yang, *J. Radioanal. Nucl. Chem. Ar.*, 1995, **173**, 195.
855. S. Anzalone, E. Bottari and M.R. Festa, *Anal. Chim. Acta*, 1997, **343**, 241.
856. S. Anzalone, E. Bottari and M.R. Festa, *Anal. Chim. Acta*, 1997, **343**, 241.
857. M.J. Anjos, R.T. Lopes, E.F.O. De Jesus, S. Moreira, R.C. Barroso and C.R.F. Castro, *Spectrochim. Acta B*, 2003, **58**, 2227.
858. O.A. Akanle, F.A. Balogun, J.A. Owa and N.M. Spyrou, *J. Radioanal. Nucl. Chem*., 2001, **249**, 71.
859. M.J.A. Armelin, V.A. Maihara, M.B.A. Vasconcellos, D.I.T. Favaro and V.F. Nascimento, *J. Radioanal. Nucl. Chem. Le*., 1992, **164**, 265.

860. F.A.R. Martino, M.L.F. Sanchez and A. Sanz-Medel, *Anal. Chim. Acta*, 2001, **442**, 191.
861. J.A. Nobrega, Y. Gelinas, A. Krushevska and R.M. Barnes, *J. Anal. At. Spectrom*., 1997, **12**, 1239.
862. H. Zhou and J.H. Liu, *At. Spectrosc*., 1997, **18**, 115.
863. F. Cubadda, A. Raggi, A. Testoni and F. Zanasi, *J. AOAC Int*., 2002, **85**, 113.
864. P. Braetter, I. Navarro Blasco, V.E. Negretti de Braetter and A. Raab, *Analyst*, 1998, **123**, 821.
865. T. Prohaska, G. Kollensperger, M. Krachler, K. De Winne, G. Stingeder and L. Moens, *J. Anal. At. Spectrom*., 2000, **15**, 335.
866. M. Krachler, T. Prohaska, G. Koellensperger, E. Rossipal and G. Stingeder, *Biol. Trace Elem. Res.*, 2000, **76**, 97.
867. F.A.R. Martino, M.L.F. Sanchez and A. Sanz-Medel, *J. Anal. At. Spectrom*., 2000, **15**, 163 .
868. A. Wagner and J. Boman, *Spectrochim. Acta B*, 2004, **59**, 1125.
869. F. Cubadda, M. Baldini, M. Carcea, L.A. Pasqui, A. Raggi and P. Stacchini, *Food Addit. Contam*., 2001, **18**, 778.
870. G. Thiel and K. Danzer, *Fresen. J. Anal. Chem*., 1997, **357**, 553.
871. L.-X. Sun, K. Danzer and G. Thiel, *Fresen. J. Anal. Chem*., 1997, **359**, 143.
872. H. El Azouzi, M.L. Cervera and M. de la Guardia, *J. Anal. At. Spectrom*., 1998, **13**, 533.
873. S.F. Durrant and N.I. Ward, *J. Micronutr. Anal*., 1989, **5**, 111.
874. A. Moreda-Pineiro, A. Fisher and S.J. Hill, *J. Food Compos. Anal*., 2003, **16**, 195.
875. J. Borkowska-Burnecka, W. Miazga and W. Zyrnicki, *Chem. Anal*. (*Warsaw*), 2000, **45**, 429.
876. J. Riondato, F. Vanhaecke, L. Moens and R. Dams, *J. Anal. At. Spectrom*., 2000, **15**, 341.
877. M. Krachler, F.S. Li, E. Rossipal and K.J. Irgolic, *J. Trace Elem. Med. Biol*., 1998, **12**, 159.
878. I. Juranovic, P. Breinhoelder and I. Steffan, *J. Anal. At. Spectrom*., 2003, **18**, 54.
879. V. Gundersen, D. McCall and I.E. Bechmann, *J. Agric. Food Chem*., 2001, **49**, 3808.
880. A. Lozak, K. Soltyk, P. Ostapczuk and Z. Fijalek, *Sci. Total Environ*., 2002, **289**, 33.
881. M. Bettinelli, U. Baroni, S. Spezia and C. Terni, *At. Spectrosc*., 2000, **21**, 195.
882. J.A. Nobrega, Y. Gelinas, A. Krushevska and R.M. Barnes, *J. Anal. At. Spectrom*., 1997, **12**, 1243.
883. M.A. Barreiros, M.L. Carvalho, M.M. Costa, M.I. Marques and M.T. Ramos, *X-Ray Spectrom*., 1997, **26**, 165.
884. J.F. Tyson, H. Ge and E.R. Denoyer, *J. Anal. At. Spectrom*., 1997, **12**, 1163.
885. L. Noel, J.C. Leblanc and T. Guerin, *Food Addit. Contam*., 2003, **20**, 44.
886. B. Maichin, M. Zischka and G. Knapp, *Anal. Bioanal. Chem*., 2003, **376**, 715.
887. S.P. Dolan and S.G. Capar, *J. Food Compos. Anal*., 2002, **15**, 593.
888. R.W. Dabeka, H.B.S. Conacher, J.F. Lawrence, W.H. Newsome, A. McKenzie, H.P. Wagner, R.K.H. Chadha and K. Pepper, *Food Addit. Contam*., 2002, **19**, 721.

889. C.M.R. Almeida, M.T.S.D. Vasconcelos, M. Barbaste and B. Medina, *Anal. Bioanal. Chem*., 2002, **374**, 314.
890. K.P. Koutzenogii, T.I. Savchenko, O.V. Chankina, G.A. Kovalskaya, L.P. Osipova and A.V. Bgatov, *J. Trace Microprobe Tech*., 2003, **21**, 311.
891. P. Kment, M. Mihaljevic, V. Ettler, O. Sebek, L. Strnad and L. Rohlova, *Food Chem*., 2005, **91**, 157.
892. A. Marcos, A. Fisher, G. Rea and S.J. Hill, *J. Anal. At. Spectrom*., 1998, **13**, 521.
893. D. Amarasiriwardena, M. Kotrebai, A. Krushevska and R.M. Barnes, *Can. J. Anal. Sci. Spectrosc*., 1997, **42**, 69.
894. M. Aceto, O. Abollino, M.C. Bruzzoniti, E. Mentasti, C. Sarzanin and M. Malandrino, *Food Addit. Contam*., 2002, **19**, 126.
895. R.M. Parr, *J. Radioanal. Nucl. Chem*., 1988, **123**, 259.
896. A. Chatt, H.S. Dang, B.B. Fong, C.K. Jayawickreme, L.S. McDowell and D.L. Pegg, *J. Radioanal. Nucl. Chem*., 1988, **124**, 65.
897. I. Rosborg, B. Nihlgard and L. Gerhardsson, *Water Air Soil Pollut*., 2003, **142**, 261.
898. J.D. Greenough, H.P. Longerich and S.E. Jackson, *Aust. J. Grape Wine Res*., 1997, **3**, 75.
899. V.F. Taylor, H.P. Longerichand J.D. Greenough, *J. Agric. Food Chem*., 2003, **51**, 856.
900. J.P. Perez-Trujillo, M. Barbaste and B. Medina, *Anal. Lett*., 2003, **36**, 679.
901. G.J. Martin, J.B. Fournier, P. Allain and Y. Mauras, *Analusis*, 1997, **25**, 7.
902. M. Barbaste, B. Medina, L. Sarabia, M.C. Ortiz and J.P. Perez-Trujillo, *Anal. Chim. Acta*, 2002, **472**, 161.
903. C.M.R. Almeida and M. Vasconcelos, *J. Agric. Food Chem*., 2003, **51**, 4788.
904. M.J. Baxter, H.M. Crews, M.J. Dennis, I. Goodall and D. Anderson, *Food Chem*., 1997, **60**, 443.
905. C. Reimann, K. Bjorvatn, B. Frengstad, Z. Melaku, R. Tekle-Haimanot and U. Siewers, *Sci. Total Environ*., 2003, **311**, 65.

Index

Page numbers followed by t refer to tabular entries; numbers followed by f denote entries in figures.