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NEUTRON ACTIVATION ANALYSIS *for* CLINICAL TRACE ELEMENT RESEARCH

Volume I

K. Heydorn

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PREFACE

The application of scientific methods to the study of health and disease has a long history, and progress in analytical chemistry has always stimulated clinical research. The interface between these two subjects is the sampling process, which, however, has attracted very little attention until recent years.

Trace element analysis is carried out routinely by a variety of methods, but the present-day challenge is the reliable determination of elements at the ultratrace level, where neutron activation analysis is superior to all other methods for many elements.

Only within the last decade have the unique advantages of NAA been fully utilized to control sampling problems and improve the quality of clinical trace element data.

This monograph is intended as a reference for prospective or existing research groups engaged in the study of elements at the ultratrace level in clinical samples. In such an interdisciplinary cooperation there is a need to bridge the gap between the analytical and the clinical scientist with information on the possibilities and the problems associated with the use of neutron activation analysis. This book is not a textbook to convert a medical doctor into an analyst or the reverse, and many subjects are only briefly introduced before being discussed, but it is to be hoped that the exchange of ideas across the interdisciplinary boundary will be stimulated.

Quality assurance of clinical trace element research cannot usually be based on the routine methods of the clinical laboratory. The small number of results characterizing the clinical research work must instead be produced by methods with few and well-known sources of variation. NAA is particularly applicable to these requirements and has been shown to be capable of yielding results in statistical control. In this book this property of NAA is the basic condition for using the Analysis of Precision to detect and identify other sources of random or systematic errors.

Only by exact control of the sampling procedure can results in statistical control be ascertained, and an entire chapter is devoted to this subject. The next chapters discuss in some detail all sources of variation associated with NAA, and it is shown that all those of importance for biological samples may be brought in statistical control, even at the ultratrace level of concentration. Neither in vivo activation analysis nor analysis by prompt gamma-ray emission are capable of producing results at such low levels, and in addition their technical sources of variation are quite different from reactor neutron activation analysis.

Sample homogeneity is taken into account as a special source of variation, separate from analytical precision, and in this way the Analysis of Precision may be used to detect and identify systematic errors and eliminate unreliable data. This methodology is developed in Chapter 5 and used in Chapter 6 to ascertain reliable data for normal levels of a number of trace elements in human tissue and blood. The identification of significant sources of variation in healthy individuals is discussed in the next chapter together with a method of detecting abnormal distributions of trace elements in the human body by multi-dimensional data analysis. The final chapter gives examples of associations between such abnormal trace element levels and various diseases.

Recent progress in this field has shown that many previous assertions were based on inadequately controlled data, and the present book is therefore almost exclusively based on the literature published in the last decade. No attempt has been made to quote all relevant papers, but to select material best suited to illustrate the main line of thought and to emphasize aspects less thoroughly covered in other monographs.

ACKNOWLEDGMENTS

During the preparation of this monograph I have been privileged to receive assistance from many individuals and organizations not only in Denmark, but in many European countries, as well as in the United States.

I am particularly grateful to friends and colleagues at the Institute of Nuclear Sciences in Ghent for allowing me to draw on their vast experience in this field, including pertinent unpublished work. I also owe a great deal to other individual scientists who volunteered to read parts of the text; their constructive criticism and valuable advice have greatly improved the quality of the book. Any lack of precision and clarity, as well as residual errors and omissions, are the sole responsibility of the author.

The permission to reproduce several illustrations and tables from other publications is gratefully acknowledged. Some of the original figures have been slightly modified, but in each case reference is made to the original publications in the figure caption. The full sources can be found in the reference lists at the end of each chapter.

Finally, I wish to acknowledge Risø National Laboratory for permission to utilize its excellent technical and personnel resources during the preparation of the manuscript.

DEDICATION

To Siri and Arne

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Chapter 1

INTRODUCTION

The application of scientific methods to the study of disease dates back several millennia to the time of Hippocrates,* and today clinical research covers an extremely wide range of activities.

Neutron activation analysis can be much more precisely dated — to the time of Hevesy, who published the first account of the method from the Niels Bohr Institute in Copenhagen in 1936.[1] Since then, this technique has developed into an important tool for scientific research in almost all conceivable areas of interest.

The common ground between the two fields is the association between trace element concentrations and disease, coupled with the growing awareness of the inadequacy of available trace element data for the healthy person. For the determination of many elements at the ultratrace level of concentration — <10 mg/m^3 — neutron activation analysis is superior not only in sensitivity and accuracy, but in addition offers a methodology to ascertain the absence of unknown errors.[2]

Neutron activation analysis (NAA) is, therefore, particularly suited to providing reliable answers in clinical research concerning the influence of trace elements on health and disease.

I. CHARACTERISTICS OF CLINICAL RESEARCH

Clinical investigations are always closely associated with the patient, and the benefit from the result must always be weighed against the discomfort of the individual. Investigations unnecessary for the individual should be carried out only when they cause no additional strain on either the patient or his relatives. In Scandinavia and many other countries, clinical research is carried out under strict observance of the recommendations in the Declaration of Helsinki.[3]

Clinical research is, therefore, different from other types of scientific research. Like astronomy it does not have the freedom to plan experiments, or, in any event, choose its own ways of studying a subject. Rather it has to make do with the study of samples and patients that are available and be denied the possibility of selecting them for some specific purpose. Many measurements have thus been made on samples taken for an unrelated purpose and under conditions that may not always be appropriate. This applies not only to blood, urine, and biopsy samples, but to samples taken by autopsy as well.

This difficulty of exercising control over the subjects under study is so characteristic of clinical research that a comprehensive control and verification of the observed data is mandatory to avoid making unwarranted conclusions of clinical importance.

A. Elemental Analysis

The determination of *major* elements like calcium, chlorine, magnesium, phosphorus, potassium, and sodium is carried out in the clinical laboratory on a routine basis, and the importance of these elements to health and disease has long been recognized.

Other elements like iron, copper, and zinc are found in much lower concentrations in the organism and could not be determined by classical chemical analysis; their presence was reported as trace. Together with other elements occurring at 0.01% or lower concentrations, they were summarily referred to as *trace* elements. Their importance is now gaining the acceptance awarded to the vitamins in the course of the first 50 years of this century. Some

* Ca. 460-377 B.C.

Table 1.1
DISCOVERY OF ESSENTIAL TRACE ELEMENTS

Iron	17th Century	
Iodine	1850	Chatin, A.
Copper	1928	Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A.
Manganese	1931	Kemmerer, A. R. and Todd, W. R.
Zinc	1934	Todd, W. R., Elvehjem, C. A., and Hart, E. B.
Cobalt	1935	Underwood, E. J. and Filmer, J. F.; Marston, H. R.; Lines, E. W.
Molybdenum	1953	deRenzo, E. C., Kaleita, E., Heytler, P., Oleson, J. J., Hutchings, B. L., and Williams, J. H.; Richert, D. A. and Westerfeld, W. W.
Selenium	1957	Schwarz, K. and Foltz, C. M.
Chromium	1959	Schwarz, K. and Mertz, W.
Tin	1970	Schwarz, K., Milne, D. B., and Vinyard, E.
Vanadium	1971	Schwarz, K. and Milne, D. B.; Hopkins, L. L. and Mohr, H. E.
Fluorine	1972	Schwarz, K. and Milne, D. B.
Silicon	1972	Schwarz, K. and Milne, D. B.; Carlisle, E. M.
Nickel	1974	Nielsen, F. H. and Ollerich, D. A.; Anke, M., Grün, M., Dittrich, G., Groppel, B. and Hennig, A.; Kirchgessner, M. and Schnegg, A.

of these elements can now be determined in the clinical laboratory with reasonable precision and accuracy.

For more than 300 years, iron has been known to be essential to life, and in 1850 Chatin found iodine to be essential by careful analysis of the correlation between low concentrations of iodine and endemic goiter. In this century, an additional 12 trace elements, listed in Table 1.1, have been found to be essential, for mammals. Many other elements are assumed to be nonessential, while some are classified as toxic. The distinction between essential, nonessential, and toxic elements is perhaps only a question of concentration. Even the elements arsenic, cadmium, and lead, all of which are toxic at fairly low concentrations, may be essential at still lower concentrations.[4]

The specific role of trace elements in biological processes is a subject of considerable, current interest.[5] An increasing number of elements are known to be crucial to enzyme functions. In metal-activated enzyme systems, the metal is loosely associated and may act as a temporary link between the enzyme and its substrate. In metallo-enzymes, a small number of metal atoms are more firmly bound to a number of specific sites.

More detailed information on the effects of individual trace elements on human health may be found in the monograph by Underwood.[6]

B. Specific Problems

The analytical problems specifically associated with clinical research are closely linked to the limited amount of sample material available, combined with the low concentrations of trace elements present.

The greatest problem, however, is perhaps the characterization and classification of the subjects under study with respect to their normal trace element status.

The *availability* of well-characterized control samples from normal subjects is most often limited. This is a consequence of the fundamental conditions pertaining to clinical research that the patient should be subjected only to investigations relevant to the proper treatment of his condition. Samples from healthy individuals, therefore, become available only when they are offered voluntarily by a fully informed person.[7]

With respect to autopsy samples, other limitations apply. In many countries, autopsy is allowed to determine only the cause of death, and gaining permission from relatives to perform additional investigations may take too long. Persons dying in a hospital are usually well characterized, but not strictly normal even in respects not associated with their disease. Presumably normal samples are most often obtained by autopsy of accident victims, but in

Table 1.2
CONCENTRATIONS OF MANGANESE IN NORMAL HUMAN SERUM IN DIFFERENT COUNTRIES

Mean value mg/m³	Coefficient of variation	Country or state	Investigator	Refs.
0.63	20%	CA	Fernandez	9
0.59	31%	NY	Cotzias	10
0.54	30%	DK	Damsgaard	11
0.57	23%	B	Versieck	12
0.58	35%	GB	Halls	13

many cases no reliable health record prior to the accident is available, and satisfactory characterization may not be possible.

Well-characterized samples from healthy persons are such a scarce commodity that they should be analyzed with the greatest care.

Also, the *quantity* of sample material is usually limited by physical, medical, or legal restrictions, or by ethical rules.[8]

For substances like sweat, saliva, and urine, the quantity of sample is limited mainly by its production; whereas for hair, nails, etc. ethical, or perhaps aesthetical, considerations determine the maximum sample size. The volume of blood or other fluids in a sample is limited by medical restrictions, so that from anemic patients, neonates, etc., only the smallest quantity may be taken.

Biopsy samples must always be kept at a minimum, and in some countries legal restrictions also limit the amount of autopsy material removed from the body.

The physical size of several human organs or anatomical structures like the lens of the eye, the pituitary gland, the pineal gland, and some of the nuclei of the brain are quite small. Also, the quantity of cerebrospinal or synovial fluid is limited.

Separation of samples such as whole blood into cellular and noncellular components further reduces the quantity available for analysis. While the total corpuscular volume of normal blood is approximately 43%, the quantities of white blood cells and blood platelets are only 0.55% and 1.05% of the total corpuscular mass, respectively.

In short, in clinical studies sample size cannot be increased to facilitate an analysis or reduce contamination. The process of sampling is, therefore, of crucial importance and will be discussed in detail in Chapter 2.

Concentrations of many trace elements are so low in biological samples that they can be detected only by the most sensitive methods. Even then, it is quite possible that they have a biological function. As an example, the element manganese is an essential element with several established functions in humans. Its concentration in human serum is only about 10^{-8} *M*;[9-13] nevertheless, this low concentration is subject to an impressive homeostatic control[10] and shows no geographical variation, as indicated in Table 1.2.[14]

As pointed out by Bowen,[15] an element with a concentration in blood of barely more than one atom per red cell could conceivably have some biological function, and this represents a limit of clinical interest of approximately 10 nmol/m³ or three orders of magnitude below the concentration of manganese in serum.

Even the hitherto lowest reported concentration[16] of an essential element in humans, 0.02 mg/m³ of vanadium in erythrocytes, corresponds to almost 20 atoms of vanadium per red cell.

Some trace elements play a role in the synthesis and structural stabilization of proteins and nucleic acids. Some are involved in the function of organized subcellular systems, in

membrane transport, nerve transmission, and muscle contraction. Toxic effects on biological systems seem to occur at biochemical loci similar to those where essential elements control the normal function. For example, trace metals may serve as enzyme inhibitors, alter membrane permeability, impair protein synthesis, or distort nucleic acid structure.[17]

It is beyond the scope of this book to present a detailed discussion of the physiological or toxicological importance of individual trace elements. Interested readers may consult recent books and monographs written about these subjects.[5-6,18-22]

C. Research Needs

Thus, in clinical research, there is an urgent need for the reliable analysis of precious samples in small quantities for low concentrations of a number of elements.

For many elements — perhaps even for the majority of them — reliable data for concentration in normal human tissue or body fluids are unavailable. In the compilation by Iyengar et al.,[23] reported concentrations for many elements of potential biological importance are upper limits only or disagree by several orders of magnitude, even for readily available materials such as blood and urine. For example, the reported levels for the essential element manganese in serum range from 0.54 to 61 mg/m^3, while only an upper limit of concentration is quoted for the toxic element thallium.

For less readily available tissue materials, the situation is correspondingly worse. This is particularly true for more complex organs as the central nervous system, where anatomically and functionally different parts need to be studied and analyzed separately.

Research in the field of trace elements has attracted only few clinicians. The plethora of unreliable data provides a fertile ground for unsubstantiated claims and counterclaims, while the difficulties in identifying the few reliable data hamper real progress in the field. Only for such elements, like copper and zinc where normal levels and ranges of concentrations in healthy persons have been established, can abnormal conditions be identified and associated with other clinical observations or symptoms.[12,24]

For these two elements, deficiencies have been demonstrated in children of several countries, including the U.S.[25] In addition, some genetic disorders have been shown to involve inborn errors of metabolism of both copper[26-28] and zinc.[29,30]

For many additional trace elements, similar investigations may be carried out by proper use of NAA in combination with a careful evaluation of all sources of variability.

II. CHARACTERISTICS OF ACTIVATION ANALYSIS

All analytical techniques used for determining the concentration of an element in a sample depend upon some characteristic property of the atomic structure of that element. Conventional methods rely ultimately on the configuration of the electron shells, that determine not only the chemical reactions, but also the optical and X-ray spectra. Activation analysis, on the other hand, is dependent on the structure of the nucleus — but independent of the chemical state of the atom.

The discovery of *activation* was made by Irène Joliot-Curie and her husband Frederic Joliot who reported in 1934 that the elements aluminum, boron, and magnesium were made radioactive by bombardment with α-particles. Many other nuclear projectiles, including neutrons, protons, deuterons, etc. were soon found to induce activity in different elements, and the application of this technique to elemental *analysis* was introduced by Georg von Hevesy and Hilde Levi in 1936,[1] using neutrons to activate dysprosium.

In general, the induced activity is proportional to the amount of element irradiated, and the constant of proportionality is determined by parameters such as the activation cross section of the element, the flux density of activating particles, the duration of irradiation, and the half-life of the *indicator* isotope. When these parameters are known, the sensitivity

of the method may be calculated, but for actual analytical investigations, a *comparator* is preferred, which is activated under the same conditions as the sample, but has a known content of the element to be determined.

The quantity of an element A to be determined is therefore calculated from the equation

$$\frac{\text{quantity of A in sample}}{\text{quantity of A in comparator}} = \frac{\text{radiation intensity of A in sample}}{\text{radiation intensity of A in comparator}}$$

The activation by particles is not very specific, and usually activity is induced not only in the *determinand* A, but also in many other elements contained in the sample. For this reason, selective methods of measurement have to be used, most often in combination with radiochemical separation.

The decisive advantage of activation analysis in comparison with other methods of trace element analysis is the absence of blank values from reagents added after the end of irradiation. This is of particular importance in analyzing biological material, where the necessary preliminary to analysis is the decomposition of a sample by the addition of relatively large amounts of reagents. Even the most carefully cleaned apparatus and purest available chemicals may contribute a larger amount of determinand as contamination than is present in the actual sample. This is of no consequence after activating the sample, when the element to be determined is radioactive, whereas the contamination from the reagents is nonactive.

This opens the possibility of deliberately adding known amounts of the elements to be determined, which may serve as chemical *carriers* in order to simplify any radiochemistry that may be applied to the sample. By measuring the recovery of added carrier, a correction for possible losses of an element during the separation may be made.

With respect to contamination prior to irradiation, activation analysis is of course just as susceptible to errors as any other analytical method; the application of the *Analysis of Precision*,[31] however, makes possible the detection of such unexpected sources of variability.

To reap the full benefit of activation analysis, the activated sample should be accessible within a short time following the irradiation, and the laboratory should be permitted to handle radioactive material at the 10 to 100 MBq level of activity. This should be possible in any modern and reasonably well-equipped, analytical chemical laboratory,[32] but most countries require a special license from the Health Protection Authority. Such a license would normally demand that rules for the safe handling of radioactive materials[33] be strictly observed and also that radiation monitoring equipment be available.

In most cases, the cost of activation analysis is much higher than those of other analytical methods, and in typical applications the time elapse between taking the sample and obtaining the final analytical result may be several days or even weeks. Such time scales severely limit the usefulness of activation techniques in clinical analysis for diagnostic purposes. For clinical research, however, obtaining a correct result should always be the overriding consideration, and therefore activation analysis may often be the method of choice, even in cases where other methods might provide a cheaper and quicker, but less accurate result.[34]

A. Methodology

A comprehensive activation analysis comprises five well-defined steps, which may be discussed separately; some of these may be omitted under suitable conditions, as illustrated in Table 1.3.

The sampling stage includes all operations carried out prior to activation and will be discussed in detail in Chapter 2. In most cases, this stage can be reduced to taking the sample and placing it in a suitable container — and even this is avoided in the so-called *in vivo activation analysis*. This is a kind of *instrumental activation analysis*, where the whole or selected parts of the body of a living person are irradiated, and the activation products identified and determined by direct measurement.

Table 1.3
ACTIVATION ANALYSIS METHODOLOGY

Type	Sampling	Irradiation	Separation	Measurement	Recovery
In vivo	–	+	–	+	–
Instrumental	+	+	–	+	–
Radiochemical	+	+	+	+	–
Comprehensive	+	+	+	+	+

Table 1.4
TYPES OF ACTIVATION USED IN THE ANALYSIS OF BIOLOGICAL MATERIALS

Activation by		Source	Characteristics	Frequency[a]
Photons		Linear accelerator Betatron	Limited access to high-energy photons and low reaction cross sections reduces applicability considerably; inadequate sensitivity for many elements	1½%
Charged particles		Cyclotron	Mainly surface analysis based on prompt radiation; applications other than for elements with low atomic number are uncommon	1%
Neutrons	Fast	Neutron generator Isotopic sources	In vivo determination of major elements and localized accumulation of some trace elements; other biological applications are rare	2%
	Slow	Nuclear reactor	Both thermal and epithermal neutron cross sections are high, but very little activity is induced in the major elements H, C, N, O; numerous trace elements of biomedical interest can be determined	95%

[a] Estimated from the review of Bowen[35] covering the period 1970—1979.

The normal in vitro instrumental activation analysis (INAA) applies to small samples which have been removed from the patient before the analysis. In many cases, the precision of a purely instrumental method is unsatisfactory, and a *radiochemical method* must be used, where a separation of the element to be determined is carried out before the measurement.

The last step in a comprehensive activation analysis is the determination of the chemical yield of the separation, usually based on the recovery of added carrier. The omission of this step defeats the advantage of achieving statistical control in nonroutine measurements, which is of particular relevance in clinical research.

1. Activation Sources

In principle, activation analysis can be carried out with neutrons, charged particles, or high-energy photons. Different types of activation require different activation sources, each with its own physical and technical characteristics. A rough comparison of the different activation methods can be made from the information presented in Table 1.4., from which it may be seen that for biological materials *neutron* activation analysis is by far the most important.

This is associated with the much lower activity induced in most elements per unit flux density of charged particles[36] and photons[37] than of neutrons with thermal and epithermal energies.

Neutron generators, as well as those isotopic sources that generate fast neutrons, may

also serve as a source of thermal neutrons, e.g., for in vivo activation; the neutron flux densities available in a nuclear reactor, however, are orders of magnitude higher and provide the greater *sensitivities* needed for the determination of trace elements at low concentrations in small samples.

With the exception of in vivo studies, it is, therefore, necessary to have access to a nuclear reactor in order to apply NAA to important problems.[34] This is not a severe limitation in contemporary clinical trace element research, which will always be carried out as a cooperative effort involving specialists in different fields.

Most research reactors have a program in activation analysis, and with approximately 180 such reactors in 45 countries,[38] it should not be difficult to establish such cooperation. This should not be limited to the carrying out of the required neutron irradiation of samples, but should encompass the entire clinical research project beginning with the taking of the sample and ending with the interpretation of the results. Only then can the quality of the work be ascertained, and the outcome of the investigations be trusted.

2. Instrumental Methods

In vivo neutron activation analysis involves an entirely different set of problems compared with conventional in vitro analysis, which are not, in general, relevant to trace element analysis. Irradiation of a subject or parts of his body is carried out by neutrons from a sealed-tube neutron generator or another suitable neutron source, and the induced activity is compared with that of an irradiated phantom of known composition. Activation by fast or slow neutrons permits the determination of whole body composition with respect to the major elements oxygen, nitrogen, calcium, phosphorus, potassium, sodium, and chlorine. Trace elements with localized, high concentrations, such as iodine in the thyroid and cadmium in the kidney, may also be determined by in vivo activation analysis.[39] Similar measurements may, however, be carried out also by conventional NAA in conjunction with the application of the occupancy principle.[40]

The irradiation dose to the patients is usually restricted to a maximum of 10 mSv — 1 rem — which is, after all, a considerable dose in comparison with the irradiation exposures causing so much concern in connection with the operation of nuclear power plants. The principle of all irradiation doses being As Low As Reasonably Achievable — ALARA[41] — puts the question of whether the benefits from the results obtained outweigh the additional radiation exposure or not. The ethical problems involved in in vivo NAA are still a subject of discussion,[42] and the relationship between precision and accuracy vs. radiation dose is not a matter of scientific evaluation only.

Neither the techniques, nor the problems involved in this special type of INAA, has any bearing on the determination of trace elements by conventional NAA. Details of the method and its applications are, therefore, beyond the scope of this monograph and will not be discussed further.

Conventional or *in vitro instrumental neutron activation analysis* (INAA), assumes the presence of a sample in such a form that after irradiation no treatment or separation is carried out before measurment of the activity induced. A comparator sample with known elementary composition is irradiated and measured in exactly the same way as the sample.

For the analysis of biological samples containing many different trace elements giving rise to many different radionuclides, measurement of the activity must be made with the highest possible *selectivity*. Some selectivity is achieved by taking into account the different half-lives of the individual radionuclides, using measurements made at different decay times after the end of irradiation. For very short half-lives, such multiscaling measurements of β-particles have been used for determining lithium[43] by decay curve resolution; for longer half-lives, it has been found that after about 3-weeks decay the emission of hard β-particles from most biological samples is almost entirely due to radioactive phosphorus, which may then be determined by a single measurement.[44,45]

The emission of neutrons from an irradiated sample indicated the presence of fission products from uranium or thorium; the use of a detector which is sensitive only to neutrons then makes possible the selective determination of these elements.[46]

For the vast majority of radionuclides emitting characteristic γ-rays, *activation spectrometry*, based on the energy-selective X- or γ-ray detection in germanium or silicon semiconductor materials, is the standard method of measurement[47] and has become almost synonymous with INAA. This method makes possible the simultaneous determination of a number of elements in a single sample, which is highly desirable in clinical investigations. It does not require extensive manual efforts; in fact, the few necessary tasks may be carried out automatically with relatively simple equipment. It is thus feasible to produce large numbers of analytical results at relatively modest cost in terms of time and effort.

Errors associated with the processing of the irradiated sample are obviously absent, and the availability of the sample at the end of the analysis for possible further investigations is an additional advantage.

One complication is the sophisticated and expensive instrumentation needed to extract and digest the huge amount of information from high-resolution detectors; a typical system may comprise the detector itself with its electronic systems, a multichannel analyzer with at least 4096 channels, and a minicomputer with a suitable program package, as well as the necessary peripheral equipment. Last, but not least, an experienced and critical analytical scientist is needed in order to avoid the large-scale dissemination of meaningless numbers.

3. Radiochemical Methods

The most important limitation of the purely instrumental method for trace element analysis is that the relatively few predominant radioisotopes in the gamma-ray spectrum of the sample obscure the contributions from less abundant indicator radionuclides. This means that many elements at low concentrations are not detected, even though their actual sensitivity is sufficiently high. In borderline cases, prolonged decay and counting times may help the situation, but the total analysis time becomes unacceptably high. In these cases, radiochemical separation is required prior to measurement of the sample.

A comprehensive description of activation analysis with radiochemical separation and yield determination was given by Boyd[48] in 1949, showing that extremely low detection limits could be combined with the highest *specificity*. This method is of much more general applicability than the purely instrumental one, but is no longer a multielement method. Much simpler and less expensive instruments are needed for measuring the radioactivity of the separated sample, and the method is usually referred to as *radiochemical neutron activation analysis*, abbreviated RNAA, with or without yield determination.

The typical separation procedure in a comprehensive NAA of a biological sample involves the following steps: sample dissolution, carrier addition, chemical processing and chemical yield determination. Dissolution may be achieved by decomposition with concentrated sulfuric acid and oxidation by nitric acid or hydrogen peroxide in an open flask. The carrier added is a known amount of the element to be determined, usually in the range of 0.1 to 20 mg, and its purpose is to increase the concentration of the determinand from its original trace level to a constant level, which is high enough both to avoid losses by adsorption, etc. and to facilitate macrochemical separation steps. It is absolutely essential that complete exchange between the activated trace element and the carrier takes place, and this is normally achieved by carrying out a complete oxidation-reduction cycle.

Radiochemical separation may now be carried out by the same techniques as are applied in conventional, inorganic trace analysis.[49,75] Some of which are listed in Table 1.5., taking into account the half-life of the indicator isotope. After measuring the radioactivity of the separated sample, the chemical yield may be determined by any of a number of analytical methods, such as gravimetry, spectrophotometry, INAA,[50] or by means of a suitable radioactive tracer added to the carrier.[51]