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NEUTRON ACTIVATION ANALYSIS *for* CLINICAL TRACE ELEMENT RESEARCH

Volume II

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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 5

ACCURACY

Like precision, accuracy is difficult to express quantitatively. Choosing as the definition, the difference between the mean value of the analytical results and the true value, it is clearly independent of precision, and the two concepts need not be confused. It is, therefore, quite possible to have poor precision and excellent accuracy or vice versa at the same time.

Accuracy is thus determined by the difference between two unknown numbers, one of which is inaccessible to measurement, the other requiring an infinity of measurements. When systematic errors are absent, this difference is zero and the accuracy is excellent.

All analytical methods worthy of consideration should account and correct for the presence of possible systematic errors, so that the expectation value of analytical results, y_{im}, is the true value, y_m,

$$\hat{y}_{im} = y_m$$

This means that the residual error, η, has an expectation value of zero,

$$\hat{\eta} = \hat{y}_{im} - y_m \tag{5.1}$$

The closest one can get to ascertaining the accuracy of analytical results is to demonstrate that under the prevailing conditions $\hat{\eta}$ is not significantly different from zero.

The sensitivity for the detection of a systematic error, i.e., a residual different from zero, depends not only on precision, but also upon the origin and types of error involved.

It is, therefore, expedient to distinguish between *systematic* errors, which affect many samples in a consistent manner, and other sources of errors, affecting *individual* samples in an unpredictable way.

Both types of error must be taken into account in the internal quality control, where verification is based on the use of alternative methodology, and the *detection of systematic errors* is based on the monitoring of the variability of duplicate results by the Analysis of Precision.

The external quality control of analytical results is based on the analysis of *reference materials*, and the most important of these should be analyzed to ascertain the absence of calibration errors.

It will be shown that analytical methods based on neutron activation need have no significant systematic errors and may be used as referee or definitive methods. At the same time, however, it is found that no analytical method, including neutron activation analysis, (NAA) is infallible, even in experienced hands.

This applies particularly to very low levels of concentration, such as is found in human blood, where statistical control cannot always be achieved. Only by careful verification and continuous control can analytical results with paramount precision and accuracy be produced at the trace or *ultratrace level* of concentration.

I. TYPES OF ERROR

Systematic errors that affect all or a large fraction of the samples contribute to the value of η in three ways:

a. A *bias*, which is independent of the concentration of determinand — but may depend on many other factors

b. A *calibration error*, which increases with concentration, approximately as a proportional error — but may depend on other factors
c. A variable error of unknown origin, but caused by factors no longer under control or unintentionally being changed

Many of the above types of error have been discussed in Chapters 2 and 3 from a technical point of view. In this chapter, methods to *verify* the accuracy of analytical results are discussed, and methods for detecting unexpected systematic errors are presented.

Gross errors that affect samples individually are usually too few to detect by a random quality control, and systems to *prevent* such errors are, therefore, warranted.

A. Systematic Errors

Instead of the word accuracy, and to avoid the same problems that were connected with precision, we may instead refer to nonrandom, systematic errors, and no quantitative definition of accuracy is then required.

Systematic errors are not reduced by increasing the number of replicate measurements, since their effect or bias, η, is measured as the signed difference between the limiting mean and the true value, as given in Equation 5.1. A special case occurs when the true value is 0; here a positive bias is usually referred to as a *blank value*.

Systematic, proportional errors, measured by the ratio of the limiting mean to the true value, are sometimes referred to as *calibration* errors. These concepts are consistent with the terms recommended by Eisenhart.[1]

Some types of error affect the analytical results proportionally without being calibration errors, and other types are quite independent of the actual concentration. It is not always obvious how a potential error is best detected, nor is it easy to identify the source of error from observed deviations from true values of reference materials or other samples with known content.

The complexity of calculation and the use of computers introduce the additional error of *calculation*, which will affect many samples. Both actual programming errors and false assumptions underlying the method of computation affect the analytical results in a systematic manner.

Verification of accuracy is based on demonstrating the absence of systematic errors, and good accuracy depends upon the application of appropriate and sensitive methods for the detection of the various types of errors.

1. Bias or Calibration

The determination of the exact magnitude of a systematic error is possible only when the true concentration is known. The bias (a) assumed to be independent of concentration is most readily determined by the analysis of materials not containing the determinand,

$$y_m \equiv 0$$

The value of $\bar{\eta}$ under these circumstances is usually referred to as a blank value, and it is determined by replicate analysis of pure materials, or by extrapolation of results from the analysis of samples with known ratios of determinand concentrations.

The significance of the blank value is tested by the statistic

$$u = \frac{\bar{\eta}}{\bar{\sigma}_0 \sqrt{N}} \tag{5.2}$$

where N is the number of replicates and $\bar{\sigma}_0$ the average standard deviation of a single result for zero concentration.

The true value is a useful concept, but it can only be approximated by actual measurement. The expectation value of all analytical results is the true value, regardless of the analytical method:

$$\hat{y}_{im}^{(1)} = y_m$$

$$\hat{y}_{jm}^{(2)} = y_m$$

This means that, when the analytical methods considered are in *statistical control*, the mean values of replicate determinations are not significantly different

$$\eta = \bar{y}_{\cdot m}^{(1)} - \bar{y}_{\cdot m}^{(2)} \tag{5.3}$$

An analytical method which gives results with insignificant systematic errors is sometimes called a *referee method* [2] or a *definitive method*.[3] It is the object of the present chapter to show how comprehensive NAA or other methods employed in trace element research may qualify as definitive methods. These methods are at the same time selected for their superior precision at very low trace element levels; comparisons with other methods are limited to the detection of calibration errors.

If η from Equation 5.3 is not significantly different from zero, all results from the two methods may be pooled to yield an overall consensus value. When the two methods are based on different analytical principles and carried out completely independently with comparable precision, their consensus value is assumed to be a practical representation of the true value.

Such considerations form the basis for the certification of *reference materials* by institutions like the National Bureau of Standards; but uncertified reference materials with a wide, international circulation may be treated in the same way without the official stamp.

Such materials are suitable for the investigation and control of calibration errors, (b)

$$\bar{\eta} = y_m^* (\bar{y}_m / y_m^* - 1) \tag{5.4}$$

but the uncertainty of the certified value y_m^* is often considerable. Reference materials are, therefore, not very suitable for the determination of bias.

In the absence of suitable certified reference materials (CRM), calibration errors may be controlled by standard addition or alternative methods[4] discussed in the next section. Other errors have to be determined by the Analysis of Precision or other statistical techniques which may be adapted to the detection of a specific error.

2. Calculation

While gross errors of calculation will be detected at an early stage of development, small but significant systematic deviations from the correct value are much more difficult to identify.

Only computer programs in statistical control can be tested for systematic errors, because only then can deviations from the true values be tested for significance, and thereby accuracy be ascertained according to Equation 5.1.

True values may be known from the addition of reference spectra by computer manipulation or by counting for accurately known times. In particular, standard reference spectra with known photopeak ratios have been made available from the International Atomic Energy Agency (IAEA)[5] and were used by Heydorn[6] to investigate the accuracy of four different computer programs for peak area evaluation (see Table 5.1.) The precision of two of these programs was ascertained in Chapter 4, Section III.B.

Table 5.1
COMPUTER PROGRAMS USED FOR PHOTOPEAK INTEGRATION AT RISØ NATIONAL LABORATORY

Program	Originator	Method of calculation			Target application
		Type	Peak	Continuum	
1	K. Heydorn[7]	Direct	PPA	*A priori* choice of integration width	Single peaks at detection limit
2	R. Gwozdz[8]	Direct	TPA	*In situ* selection of peak boundaries	Universal application
3	S. Nielsen[9]	Fitting	Simple Gaussian	Linear baseline	Poor counting statistics
4	J. T. Routti[10]	Fitting	Modified Gaussian	Parabolic baseline	Resolution of complex peaks
5	M. J. Koskelo[11]	Revised			

Table 5.2
COMPARISON OF ACCURACY OF DIFFERENT PHOTOPEAK INTEGRATION PROGRAMS

Parameter	Value	m	$P(\chi^2 \geq X)$
X_{11}	16.7	20	0.67
X_{22}	17.9	19	0.53
X_{55}	26.6	22	0.23
X_{12}	20.0	19	0.39
X_{21}	16.8	19	0.61
X_{31}	51.2	20	<0.001
X_{41}	9.3	20	>0.95

A *Test of Accuracy* is used to verify the absence of significant deviations between the true ratios, μ, given by the IAEA and the ratio of the weighted mean, $\hat{\mu}$, of the six replicates of each photopeak to the comparator,

$$X = \sum_{1}^{m} \frac{(\hat{\mu}_j - \mu_j)^2}{\hat{\sigma}_j^2}$$

$$\frac{1}{\sigma_j^2} = w_j = \sum_{i}^{6} w_{ij} \tag{5.5}$$

This weighted sum of the squares of the deviations from the true values follows a chi-square distribution with *m* degrees of freedom. Programs giving unbiased estimates of the true ratios for all m = 20 known photopeaks, are shown in the first lines of Table 5.2.

We may now compare the precision and accuracy of other programs with a particular program by means of a mixed term calculation of X

$$X_{AB} = \sum_{1}^{m} (\hat{\mu}_j^{(A)} - \mu_j)^2 \, w_j^{(B)} \tag{5.6}$$

For A = B the X_{AB} is the parameter X of Equation 5.5 used to ascertain the accuracy of the program. For both A and B unbiased, the mixed parameter makes it possible to