

Methodicum Chimicum

A Critical Survey of Proven Methods
and Their Application in Chemistry,
Natural Science, and Medicine

Editor-in-Chief

Friedhelm Korte

Volume 1

Analytical Methods

Part A

Purification, Wet Processes
Determination of Structure

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Volume 1

Analytical Methods

Part A

Purification, Wet Processes Determination of Structure

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Preface of the Series

The **METHODICUM CHIMICUM** is a short critical description of chemical methods applied in scientific research and practice. It is particularly aimed at chemists as well as scientists working in associated areas including medicine who make use of chemical methods to solve their 'interrelated' problems.

Considering the present development of science and the necessity for concise and unambiguous information, the series provides a guide to rapid and reliable detection of the method suitable for the solution of the problem concerned. Thus, particular emphasis is placed on the description of proved procedure whereby a complete and exhaustive compilation of all reported methods and also a detailed description of experimental techniques have been deliberately omitted. Newer methods as well as those which have not yet been reported in review articles are treated more extensively, whereas conventional methods are dealt with concisely. Biological procedures which, in specific cases, are more will be discussed in the analytical volume. The interrelated methods and concepts which are constantly gaining importance will be fully discussed in the third 'Specific Part'.

The **METHODICUM CHIMICUM** is comprised of three parts. The first, the 'General Part' consists of Volumes 1, 2 and 3. Volume 1 (Analytical Methods) is concerned with chemical, physical, and biological analytical methods including those necessary for the elucidation of structures of compounds.

Volume 2 (Planning of Syntheses) contains a review on fundamentals, principles, and models with particular respect to the concepts and applications of theoretical chemistry essential to the practically working scientist.

Volume 3 (Types of Reactions) is designed to illustrate the scope and utility of proved working techniques and syntheses.

The second part (Vols. 4–8), which is particularly devoted to 'Systematic Syntheses', deals with proved methods for syntheses of specific compounds. These procedures are classified according to functional groups linked together in the last step of reaction.

Volume 4 (Syntheses of Skeletons) describes the construction of hydrocarbons and heterocyclic compounds.

Volume 5 the formation of C–O-bonds, Volume 6 the formation of C–N-bonds, Volume 7 the syntheses of compounds containing main group elements, and Volume 8 compounds containing transition metal elements.

The third 'Special Part' (Volume 9–11) is concerned with the chemical aspects connected with the formulation of a question or problem.

Volume 9 deals with nonmetallic synthetic fibers and synthetic materials as well as their additives, Volume 10 with synthetic compounds and Volume 11 with natural products and natural occurring compounds.

All Volumes should not contain more than 900 printed pages. They are intended to give the chemist and any person working in fields related of chemistry a sufficient answer to his problem. Selected review articles or important original works are cited for the sake of detailed information.

We wish to thank the Georg Thieme Verlag, Stuttgart, for making possible the realization of the basic concept of **METHODICUM CHIMICUM** and for the excellent presentation of the work.

Bonn, September 1974

Friedhelm Korte

Preface of Volume 1

Volume 1 of *METHODICUM CHIMICUM* is devoted to analytical methods and techniques of compound separation which are of interest not only to the chemist but also to the scientists who works in associated areas, including medicine.

This compilation contains a short discussion of the principles of well-proved procedures so that the experienced chemist can apply them without the use of secondary literature (e.g. Chapter 3). Theory and practical techniques of the newer methods (e.g. Chapter 7) or procedures (e.g. Chapter 5, 6, 9, 14), which are rapid development, are discussed in more detail. Sufficient room is also given to those contributions which present a novel aspect to a problem (e.g. Chapters 1, 3.1, 5.2).

We considered it necessary to include the application of various methods of trace analysis to organic materials (see Chapter 10) and selected methods for the analysis of significant technical products (see Chapter 11). A critical survey of procedures for the determination of carbohydrates, proteins and nucleic acids is also given (see Chapter 12).

It is well known that the investigation of enzymatic and microbiological methods is essential in modern chemical analysis. Furthermore, techniques for the determination of chemicals in animals and higher plants (see Chapter 13.3 and 13.4) as well as methods for the biochemical transformation of

compounds of pharmacological and toxicological interest are treated. In the whole volume, the literature has been surveyed up to 1. January 1972.

We are very grateful to all authors and collaborators for their cooperation and their many valuable suggestions. The publication of the first volume has been somewhat delayed. Thanks to the close collaboration of all concerned, it has been possible to include all new significant results. Further volumes will be published in rapid succession.

We hope that this volume will convince the reader that the objective of the *METHODICUM CHIMICUM* is to provide the chemist and the scientist working in related areas of chemistry with a rapid and reliable source of information on the method applicable to his specific problem. This applies both to the whole chemistry and all the other fields of science including medicine which are closely connected with chemistry. The disposition of *METHODICUM CHIMICUM* fully considers this interrelated character.

I am particularly grateful to Professor Otto Hockwin who has given me valuable support during the copy editing of the first volume and who will also advise us in the planning and coordination of the future volumes.

Bonn, September 1974

Friedhelm Korte

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1 Foundations for the Critical Discussion of Analytical Methods

Contributed by

Heinrich Kaiser, Dortmund

Preliminary Note

In the following chapter the principles pertaining to chemical analysis are critically observed, partially from a new point of view. Therefore, some of the concepts, relationships, and words used may appear unfamiliar to the reader accustomed to the normal chemical literature. However, the adequate presentation of the ideas has required the use of special terms. Their choice was not made haphazardly from a stock of appropriate

synonyms, but by careful selection from mathematics, physics, and from the colloquial language. To assist the reader to grasp the rather abstract ideas and to stimulate his imagination, words with somewhat 'plastic' meanings were chosen. However, they have been given a precise meaning in this text. It is best to take them literally in their original sense and to transfer their understanding as far as possible into the field of the new relationships.

1.1 Basic Concepts

1 Introduction

Chemical Analyses are information processes planned — in most cases fairly well — with the aim of obtaining knowledge about the composition of the substance under investigation. For assessment and evaluation of such processes and of the information provided by them, two essentially different aspects must be considered: content and form.

A critical discussion of the content is possible only in the context of a given individual case, rather than as a generality: for instance, the content of a formally simple analysis may be of utmost importance in a trial in a court of law, for commercial negotiations, or for solution of a scientific problem. However, information processes and the information arising from them always have a formal structure, upon which the type, the scope and the reliability of the obtainable information depend; these questions can be treated in a general way within the framework of a theory. When such a theory is applied to an individual case, it may produce the basis for assessment of the content and the importance of the reported results.

The formal structure of an information process also determines the structure of the information in the sense of knowledge which is produced by this process. For chemical analyses, this means: the questions of importance regarding the type, scope, and reliability of analytical information, and the questions of *accuracy, precision, power of detection, sensitivity, specificity* and *selectivity* all of which relate to the analytical procedure as such. All these concepts, used here in their colloquial sense, if exactly defined, lead to *figures of merit* for analytical procedures. Only from these is it possible to arrive at a critical conclusion regarding analytical results which have been produced by a definite analytical procedure (cf. Section 1.2:6).

2 Concept of a 'Complete Analytical Procedure'

Figures of merit in information theory must be capable of being stated in an objective way. They can, therefore, be given only in relation to concrete analytical procedures, not to general analytical principles, such as titrimetry, spectrometry, polarography, neutron activation, etc. Figures of merit for an analysis always relate only to a definite *complete analytical procedure*, which is specified, in every detail, by fixed working directions (order of analysis) and which is used for a particular analytical task.

For a complete analytical procedure, everything must be predetermined: the analytical task, the apparatus, the external conditions, the experi-

mental procedure, the evaluation and the calibration. If any feature is altered, a different analytical procedure results.

If, for instance, quartz vessels are used instead of glass vessels, or if normal distilled water is replaced by doubly-distilled water, or if one moves to another laboratory with air conditioning and clean air, then one may have a new (and probably better) analytical procedure, in spite of the fact that the general mode of operation has remained unchanged.

One of the most effective means of achieving higher precision in chemical analysis is to take the average of the values from a number of repeated analyses. However, even this alteration of the evaluation process constitutes a change in the analytical procedure. The same would apply if the measurement period were extended (equivalent to better time averaging), for example in X-ray fluorescence analysis.

3 Structure of Analytical Procedures, Classes of Measurable Quantities

Chemical analytical procedures are a special group of technical and scientific *measurement procedures*. Very little attention has hitherto been paid to their logical structure, because interest has been concentrated on the chemical reactions and the operations to be carried out.

Any measurement procedure is embodied in the experimental setup, the instruments, substances, etc., and also in the relevant directions, which must specify the procedure in every detail.

If a measurement procedure is employed in a particular case, it produces a *measured value* x (sometimes for short called a *measure*) for the measurable quantity in question. The aim of the measurement is to find the *true value* of the measurable quantity. However, the 'measure' may deviate to some extent from the unknown true value. The difference (measure minus true value) is the *error* of this particular experimental result. It would be possible to determine how large or small the error is in a particular case only if, in addition to the measured value, the true value were also known in some way. Such a case is, however, only of theoretical interest since a measurement is normally made because one wishes to know the so far unknown true value of the measurable quantity.

Chemical analyses are carried out in order to determine the *concentrations* (symbol: c) of the substances under study, or their *absolute quantities* (q), and often the ratios (c_r or q_r) of such quantities. Various units for these quantities are used, depending on circumstances and practice; it is, therefore, essential always to indicate which

units are used, in order to avoid misunderstandings.

[Symbols in formulae stand for the measurable quantities, and always denote the product of a numerical value and the appropriate unit.]

This class of measurable quantities is the class of the *target quantities* of the analysis. To make the following considerations more concrete, we shall apply the term *content* to all target quantities, and will use the symbol *c* for them.

Chemical contents cannot be measured directly; their numerical values, the 'results of an analysis', are derived from measurements of other quantities such as weight, volume, current strength, refractive index, absorbance (extinction in German), intensity of a spectral line, pulse rate, photographic blackening etc. For these measurable quantities, which relate to the very core of a procedure, the term *measure* (symbol: *x*) should be reserved in its specific sense. What may be considered as the 'core' of an analytical procedure is often indicated by the type designation, for instance one speaks of gravimetric, polarographic, chromatographic, spectrochemical, and neutron activation analysis.

The measurable quantities which are of decisive importance in analysis are nowadays almost exclusively physical in character. However, in many cases these measures are not observed directly, but are derived from the 'reading' of an indicating instrument.

Very often this is a geometrical quantity, for instance the position of a needle, or a recorder trace, or the print-out from a digital voltmeter, etc. This is so obvious that one often overlooks the fact that the 'indicated quantities' are necessary intermediate steps, mainly because most instruments are calibrated to give numer-

ical values of the important physical quantities. For this reason, the intermediate steps, such as reading the position of a needle, will not be considered separately in the following text. It must not, however, be forgotten that indicating instruments may limit the power of a measurement procedure by having too low a precision, too restricted a range of measurement, or too high a 'reading threshold'.

Besides the two quantitative classes of decisive importance, considered thus far, the contents *c* and the measured quantities *x* (including the readings, etc.), there is a third class of quantities, the *parameters* (symbol: *r*) of the analytical procedure. The parameters (i.e. temperature, pressure, concentration of chemical reagents, pH value, wavelength in a spectrum, time) determine the conditions under which the measurements are made during the analysis.

The values of the parameters can be measured; they are often given by the external conditions (e.g. room temperature, atmospheric pressure), and in many cases they can be preset (e.g. pH value, reaction temperature, wavelength). Parameters may be adjusted to fixed values, or they may be varied over wide ranges during an analysis. Unnoticed changes of experimental parameters are often the cause of analytical errors which may appear to be accidental, but which in fact are systematic in nature and, therefore, can be corrected for if the appropriate parameter is measured (e.g. fluctuations in temperature, varying background in a spectrum). This is often disregarded; consequently, all directions relating to procedure should state specifically which parameters are important, and to which values they should be adjusted.

The various quantities, contents *c*, measures *x*, and parameters *r* are interdependent through a system of empirical correlations, theoretically derived functions, systems of equations, diagrams, etc.

1.2 Aspects of the Assessment of Analytical Procedures

1 Calibration

The relationship between measures *x* (weight, volume, current strength, intensity, etc.) and the desired values for the contents *c* is described by the 'analytical function', which must be regarded as an essential component of an analytical procedure. If this function is very simple, for instance if it is given by a stoichiometric conversion factor, its involvement may go unnoticed. However, many problems of chemical analysis cannot be understood correctly if one forgets that the link between the measure and the content is always the analytical function.

If one wishes to rely upon stoichiometric relationships or upon theoretical derivations, it is essential to have tested whether the reactions, which have been presupposed as the basis of the calculations, proceed completely and undisturbed. This is why, in publications on new analytical procedures, tables are very often given comparing the 'given' chemical contents with those 'found' by analysis. When there are systematic deviations between the 'given' and the 'found' values, these can be corrected by establishing a so-called 'empirical factor'. This procedure is none other than a calibration of the analytical procedure with standard

samples of known composition, even if not so named.

Calibration is a necessary operation when building up a quantitative analytical procedure. There are no analytical procedures which are 'absolute' in the true sense of this word, even if some are so called. To analyze absolutely — i.e. without any presuppositions — it would be necessary to identify the atoms and the molecules of the substances to be determined, to sort them out, and to count them individually and completely.

During any analysis, the sample under investigation is compared with the standard samples which were used to calibrate the analytical procedure. This is done by using the relevant 'analytical function' (which sometimes may be given only by a conversion factor). The calibration of many of the common, well proved procedures may have taken place a long time ago; the author and his paper may be forgotten; his results are, however, part of the anonymous treasure of experience in Analytical Chemistry. Every analysis, therefore, contains comparison as a basic operation in its structure. However, comparison of different objects is possible only if they are in some way 'alike' — this general concept of likeness must be specified for each particular case.

The samples submitted for analysis, and the standard samples used for calibration of the analytical procedure, must be of the 'same kind' with respect to all operations, reactions and measurements to which they are submitted during the course of the 'particular complete analytical procedure': they must belong to the same 'family' of analytical samples.

2 Classification of Calibration Methods

The method by which an analytical procedure is calibrated is an important characteristic for its assessment. The accuracy (see Section 1.2:6) of the analytical results, the range of applicability of the procedure, and the instrumental and running costs are partly dependent on the method of calibration which was used. The various methods for calibration of analytical procedures may be classified in order of decreasing effectiveness; in the following text, they are indicated by small Greek letters.

2.1 Complete Calibration Methods

2.1.1 σ -Calibration (with Synthetic Standard Samples)

Standard samples* which have been synthesized from pure substances — together with the calibration functions based on them — are in their entirety the supporting structure of Analytical Chemistry. During the course of a long historical development, these samples have been made more and

more reliable by many cross-checks, controls and corrections. Today, we are able to prepare many substances in a pure state as starting materials for the calibration of analytical procedures; in particular, we have learned to determine quantitatively how pure these substances are.

When it is possible to prepare standard samples in a reliable way, for a particular analytical procedure from pure substances, then an analytical function can be established which has no bias; and subsequent analytical results will, consequently, be free from systematic errors. Since the true contents of the standard samples are known from their composition, they can be directly correlated with the measured quantities; this allows the inevitable accidental errors of the individual calibration measurements to be eliminated by taking the average of a large number of such measurements.

σ -Calibrations are found everywhere in the practice of chemical analysis; many of the stoichiometric conversion factors in the literature have been determined in this way — mostly, however, for ideal analytical conditions. Standard samples for σ -calibrations can be prepared with reasonable expenditure only for relatively simple analytical problems, for which the type of the sample and of the components to be determined is known. Furthermore, only a few components should be involved or if many components are involved lower analytical precision should be regarded as adequate. Examples are: calibration solutions for *flame spectrometric methods*, mixtures of gases, and simple metallic alloys with known and clear phase diagrams.

In analytical organic chemistry, and particularly in biochemistry, it may be difficult to obtain the starting materials. If traces of contaminants are to be determined in substances of high purity, it may be impossible to obtain these substances free from these contaminations; they cannot, therefore, be used to synthesize σ -standard samples.

Even when synthetic samples have the correct composition, it may not be feasible to transfer them into the same physical or chemical state as the samples for analysis; this means that the standard samples and the analytical samples are not of the same kind with respect to the analytical procedure, and cannot, therefore, be directly compared.

In practice, direct σ -calibration of a complex analytical procedure is the exception rather than the rule. 'Accurate analytical' results are, however, to be expected only if they relate directly or indirectly to σ -calibrations. This is achieved with the next class, α -calibration.

* The term 'standard sample' follows the present official nomenclature. However, there is a strong movement to restrict the use of the term 'standard' to samples which have been officially analyzed and issued by some authoritative organization. It has been proposed to use the term 'reference sample' or 'calibration sample' for such material coming from other sources without an official certificate of composition.

2.1.2 α -Calibration (with Analyzed Standard Samples)

This type of calibration is especially important for analytical procedures used for complex analyses of large series of similar samples, for instance for production control in steel mills. α -Calibration proceeds in a way converse to synthetic calibration; it starts with the selection of a set of homogeneous samples which cover the whole range of the compositions in question. Tests must be carried out to determine whether the desired range is covered, and whether these selected samples are of the 'same kind' as the other samples: belong to the same 'family'. Such tests can often be carried out by using the measured value given by the uncalibrated procedure, or by performing auxiliary experiments (e.g. investigation of the crystal structure). In other cases a thorough knowledge of the task may be helpful.

The second step of an α -calibration is the analysis of the selected samples by another analytical procedure which has been σ -calibrated. There are two different ways of achieving this goal: either all samples involved must be treated in such a way that ultimately they are of the same kind with respect to the analytical procedure used for the σ -calibration, or the total analytical procedure must be split up into a number of different parts.

The first way is feasible only with relatively simple analytical procedures. For instance, metallic samples which are to be used as standards for rapid spectrochemical analysis of solid cast samples may be dissolved and then compared with σ -standard solutions made from pure salts of the elements to be determined. The second way involves more work, but is more practicable. The sample for analysis is selectively subdivided into portions of different composition by a series of separation operations, in such a way that each of the final preparations contains only one or a few of the components. Their contents can then be determined by relatively simple reactions. At the end of such an analytical procedure, the α -calibrations for the different components occur independently of each other. (A prototype of such a procedure is the classical inorganic analysis by a sequence of precipitations.) The topological scheme for such a multi-branched method of analysis is the 'tree' (see 1.2:3.1, Fig. 1. A).

It may be very difficult to get reliable results from a highly branched method of analysis. Incomplete separations may lead to systematic errors; and also, the random errors of the results will, in the end, become systematic analytical errors of the subsidiary α -calibrated procedure. This possible transfer of errors to all subsidiary analyses requires the utmost care. Checks by parallel analyses using other procedures and in other laboratories must be made. Analyzed standard samples of good reliability are generally expensive, and are commercially available only for the most important technical analytical purposes.

If standard samples are required for the determination of *chemical compounds* rather than elements, it may be very difficult or even impossible to design separation processes for the analysis of these standard samples such that the analytical information is not partly lost during the course of the operations. This is especially true for sensitive organic compounds and natural products, which are easily destroyed. In order to draw conclusions about what the original substance may have been, one must try to obtain as many relationships as possible between the observed fragments and the measured quantities in the same way as when elucidating a chemical structure. The topological scheme for such an analytical procedure is the 'network' (see 1.2:3.2, Fig. 1, N).

2.1.3 δ -Calibration (by Differential Additions)

In many cases, an analytical procedure can be calibrated by adding small but known amounts of the component to be determined to the sample undergoing analysis. This is like scanning the analytical function in small differential steps. In order to obtain a smooth and simple function, it is necessary to make a suitable choice for the measurable quantity, and to eliminate the influence of interfering parameters by applying corrections (e.g. corrections for blank values in chemical reactions, background in a spectrum, distortions of recorded traces). If the result is a calibration function with an easily apparent form, then it may be possible to extrapolate this function beyond the range which was covered by the additions, and thus to determine the unknown content which was originally present in the analytical sample.

This δ -calibration procedure, sometimes called the *addition method of calibration*, is the only one which allows quantitative determination of very small trace amounts when the basic material of the analytical sample cannot be obtained completely free from impurities^{1,2}.

The δ -calibration procedure presupposes that the added amount of the component to be determined behaves analytically in the same way as that part of the component which was originally present in the sample (the standard sample and the analytical sample must be of the same kind).

An example of how difficult the task can be is the analysis of hard refractory ceramics, such as Al_2O_3 , for traces of alkalis. The alkali atoms which are added do not enter the crystal lattice; the atoms in the lattice do

¹ G. Ehrlich, R. Gerbatsch, Z. Anal. Chem. 209, 35 (1965).

² F. Rosendahl, Spectrochim. Acta 10, 201 (1957/58).

not come out³. If the sample is dissolved or melted, contamination or losses are probable, and, if the sample is diluted during the course of such operations, the detection limit for traces may become undesirably high.

2.1.4 θ -Calibration (on a Theoretical Basis)

With some reservations, it is also possible to include in this group of complete calibration methods those which derive the contents to be determined from the measured quantities by using quite general principles and known data. Such laws, for instance, include the law of mass action, the Lambert-Beer law, the Boltzmann distribution, etc.

General laws are, of course, idealizations; they are valid only if definite assumptions are correct. In most cases they have been found and verified only under special, very pure experimental conditions. 'Pure' conditions are, under the practical conditions of chemical analysis, nearly a *contradictio in adjecto*. Interfering factors of all kinds must be taken into consideration. Factors of yield or activities demonstrate clearly that pure theory is often not sufficient. Even in cases where a theoretically derived relationship has been established and has long been proved, its validity has in most cases been verified by the use of synthesized standard samples (for instance for many analytical reactions which follow the theoretically predicted stoichiometric relationships).

However, θ -calibrations in the two meanings of this term are of importance for new analytical procedures. They provide a first approximation, until they are either verified or replaced by σ -calibrations. They often lead to analytical results which are at least almost correct; in this sense, θ -calibrations represent a transition to the next group.

2.2 Abridged Calibration Methods

2.2.1 κ -Calibration (by Convention)

Very often the results of chemical analyses provide a basis for decisions in industry, commerce, or in legal affairs. In such cases, the chemical composition of a substance may be of interest only in so far as it determines the required qualities. When technical requirements as to quality and the conditions of delivery are fulfilled, and the prescribed tolerances are observed, then one may be able to manage without knowing the 'true contents'. On the other hand, it may be necessary to state differences or ratios exactly, in order to ensure that quality specifications are fulfilled, or to decide a dispute. In such cases, the partners must agree, by convention, on a procedure which leads to directly comparable results. There are two possibilities for reaching such a procedure: either one agrees upon an 'umpire assay', including fixed calibration factors, or one calibrates the analyses by using 'official standard samples'.

³ H. Waechter in E. Rexer, Reinststoffprobleme, Bd. II — Reinststoffanalytik, p. 245, Akademie Verlag, Berlin 1966.

The convention is then to accept the results of such umpire analyses as correct within the limits set by the inevitable random errors. This assumption may be true in many cases; every effort is made to select optimal procedures for arbitration and standard samples; however, it is essential that one need not check the validity of the assumption for each single case, but rather appeal to the convention.

2.2.2 β -Calibration (Broad Band Calibration)

This term is used for analytical procedures for which it is known in advance that the calibration will not give a high analytical precision.

Instead of a calibration curve (with relatively small scatter), the relationship between measurable quantity x and content c (see 1.1:3) is represented by a broader band.

One may be compelled to forego analytical precision because this is no longer attainable; there are, however, many analytical problems whose solution does not require very high precision. Examples include many procedures for survey analysis, where classification of the contents according to their orders of magnitude may be sufficient. Such analytical procedures are sometimes termed 'semi-quantitative'. The costs of instruments and time are generally much smaller than with highly refined precision procedures. Furthermore, the problem of ensuring that the analytical samples and the standard samples are of the same kind is considerably simplified. In the broad band which represents the calibration function, the systematic errors (see 1.2:6) which are due to the different composition of the samples disappear at least partially. They can now be regarded as random errors caused by accidental differences in the composition of the samples. This opens the way to 'universal analytical procedures'. The smaller the requirements for precision and limits of detection are, the 'more universal' such procedures may be as regards the nature of the samples.

2.3 Calibration with Auxiliary Scales

For all the classes of calibration methods mentioned above, it is, in principle, possible to give the 'target quantities' of the analysis (contents, concentrations, absolute quantities) in SI units, i.e. in kg, m and mol. However, this presupposes that the nature of the substances to be determined is known, i.e. that their density or their molecular weight can be determined. If this is not the case, units or suitable scales must be introduced in an ad hoc manner.

⁴ Ch. E. Harvey, Semiquantitative Spectrochemistry, Applied Research Laboratories Inc., Glendale Cal. 1964.

2.3.1 ω -Calibration (with Agreed Units)

ω -Calibrations are often found in biochemical or pharmacological analyses. The interest may, for example, be in a substance, such as a hormone, an enzyme or a poison, principally as regards its biological effect, which is usually dependent on the quantity of substance present. In such cases, one attempts to find an analytical measure, for instance the color or turbidity of a solution, which can be attributed to the active component of the substance. In order to be able to derive from such an observation a useful value for the quantity present, the procedure must be calibrated with some 'standard sample', whose content has been determined in some arbitrarily fixed unit derived from the observed biological effect. Of this type are, for instance, the *international units* of antibiotics, or the *LD50* values (lethal dose) used in the case of poisons. The logical structure of the ω -calibration, therefore, corresponds to an α -calibration, with the difference that the basic unit is specific and valid only for the substance in question.

2.3.2 τ -Calibration (with Technical Scales)

In calibration with technical scales, no attempt is made to give any value for contents; instead, the analytical measures are directly correlated with characteristics or properties of technical or practical importance. This is possible when raw materials, chemicals, metallic alloys, etc. are to be classified. In general, only a very rough subdivision into different classes is necessary, i.e. a broad-band calibration is involved.

2.4 Stability of Calibration

The question of how long and under what circumstances the calibration values derived for a definite analytical procedure remain valid, has always been of importance for the critical assessment of a procedure and of the analytical results produced by it. Recently this question has reemerged in a hidden form, with the catch-phrase 'inter-laboratory reproducibility' which is used in the statistical treatment of series of analyses. Obviously it has not hitherto been realized that this is not so much a question of analytical precision, but rather a question of successful calibration.

Stability of calibration involves two questions: How 'robust' is the structure of an analytical procedure towards variations of the experimental parameters? To what extent has one control over an analytical procedure, as regards theory and technique, in order to keep the experimental conditions constant or to take into account the influence

of changing parameters by suitable measurements and corrections?

Let us consider a system of separated analytical functions, which may also be dependent on various parameters r_h , $h = 1 \dots j$; then a procedure may be termed 'robust' if the partial differential quotients

$$\frac{\partial f_i}{\partial r_h} \quad i = 1, \dots, n; h = 1, \dots, j$$

are small throughout the whole range of application; at best, they would be zero. This may be achieved most nearly with analytical procedures whose structure is simple, and in which only a few variables, a few parameters, and relatively simple operations are involved.

Complex analytical procedures are in most cases not 'robust', because not all sensitivities towards parameter variations,

$$\frac{\partial f_i}{\partial r_h}$$

can be made small simultaneously and throughout the whole range. For assessment of analytical procedures as regards stability, it is useful to distinguish three degrees of calibration stability.

2.4.1 Perfect Calibration (p)

The highest degree of calibration stability exists if it is possible to describe the analytical procedure sufficiently precisely, i.e. to prescribe every detail, so that it can be reproduced anywhere and at any time in such a way that the calibration function, once established, can be accepted. Analytical procedures with such a generally transferable calibration should be called perfectly or permanently calibrated (symbol: p).

Perfectly calibrated in this sense are many of the classical *precipitation reactions*, as well as analytical procedures using *titrimetry*, *coulometry* and *spectrophotometry*. Most of these are relatively simple procedures as regards task and operation. A complex analytical procedure which is composed of perfectly calibrated subsidiary procedures may not as a whole be perfectly calibrated, because the transitions between the component parts of the total procedure (for instance preliminary separations) may not be totally reproducible. On the other hand, it should be mentioned that very complex analytical procedures do exist which can be perfectly calibrated.

2.4.2 Fixed Calibration (f)

The second degree of calibration stability exists when the calibration values, once derived, cannot be generally transferred, but remain valid for a

definite experimental arrangement, a type of instrument or even an individual instrument. A necessary condition for this degree of stability is that attention is paid to the 'environmental parameters' (pressure, temperature, humidity and cleanness of the air), and also to the identity of chemical reagents originating from different sources. Analytical procedures for which the calibration validity is restricted to definite conditions and experimental arrangements should be termed firmly calibrated (symbol: f) or 'fixed' in calibration. Most analytical procedures used in routine production control are firmly calibrated.

In production control laboratories where many analyses are made with firmly calibrated procedures, it is the custom to check the calibration from time to time, for instance every day, with the aid of analysis control samples. This must be considered as a summary inspection of the procedure and the apparatus, but not as a 'control calibration'. A new calibration requires much more work, and should not be necessary for a procedure with sufficient calibration stability.

2.4.3 Calibration Linked to 'Leader Standard Samples' (I)

For many types of analysis, it is not possible to keep the experimental conditions constant over a relatively long time. To calibrate such procedures of limited stability, it is necessary to include measurements on the standard samples in each particular analysis; these standard samples lead the analysis samples through the whole procedure, and are called leader standard samples, the analytical procedures being designated 'calibrated with leader samples' (symbol: l).

2.4.4 Uncertain Calibration (u?)

Characteristic difficulties may be observed when new types of analytical procedures are being developed; at this stage, the new procedures are not yet 'complete'. The cause of large variations in the calibration function is generally some kind of factor concerned with yield, which depends greatly on the experimental conditions. What is missing, then, is a reference quantity which runs through the whole procedure and which is influenced in the same way as the quantity to be measured.

A classical example of a procedure without a definite calibration function is the 'ultimate line' technique of de Gramont, used in spectrochemistry. It has been superseded by the 'homologous line pair' technique of Gerlach, in which the analysis line is compared with one or more lines of a reference element⁵.

⁵ W. Gerlach, Z. Anorg. Chem. 142, 383 (1925).

3 Topology of Analytical Procedures

The logical interrelation of operations and decisions which, in the course of an analysis, lead from the analytical sample to the analytical result, can be represented in topological diagrams (Fig. 1). These diagrams are called 'topological' because only the general schemes of the interrelations are given, without any metrical aspect.

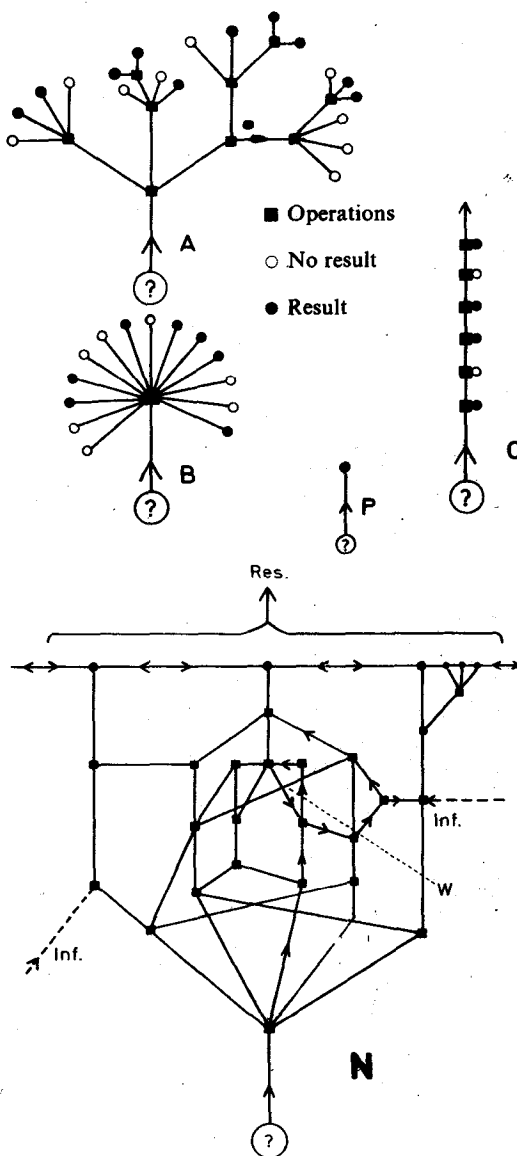


Fig. 1. Topological structure of analytical procedures
A = tree (arbor)
B = bundle (local)
C = chain (temporal)
P = point
N = net; Inf = information; W = loop; Res = total result