

THE PRESENT STATUS OF ORGANIC ELEMENTAL MICROANALYSIS

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ABSTRACT

The influence of instrumental analysis, *i.e.*, physicochemical methods, has become increasingly important in organic chemistry, but organic elemental microanalysis is by no means an obsolete field. On the contrary, with the development of new methods, the organic chemist obtains the results that he needs for a first orientation on a compound, more quickly and more reliably than ever before. Today, there are two main directions of development. First, there are the so-called classical micromethods which are used on the milligram scale. Here the trends are towards (a) the automation or instrumentation of the 'old' methods, which is normally accompanied by a significant decrease in the total time required per analysis, and (b) the employment of new principles for the determination of the final products of combustion. Secondly, there is a strong tendency to develop methods in which samples weighing less than 1 mg are used for each analysis; here instrumental methods of completing the determinations are generally applied. The importance of the determination of functional groups to organic chemists has decreased considerably in recent years because more extensive and detailed information can be obtained from the physicochemical techniques.

INTRODUCTION

Organic elemental microanalysis, as it is at present applied, is very different from the methods which Pregl developed at the beginning of this century. The original Pregl procedures that were used in the first decades of this century required considerable skill and also chemical knowledge—in these days very often the heads of chemical departments or senior assistants carried out elemental analyses; in the last two decades the methods have been perfected to such an extent that, with some few exceptions, nowadays nearly every person can be trained to analyze satisfactorily. Since it can be assumed that the historical background and the development of organic microanalysis until 1960 are well known, only a survey of the progress made in recent years, an assessment of the present status and a forecast of the future prospects will be given in the following paragraphs.

The present status of organic elemental microanalysis will be discussed on the basis of a more or less arbitrary classification. During the past few years, three main directions of development have appeared: (i) the modernization of the so-called classical methods to a degree that these methods are classical only

with respect to the amount of sample taken for one analysis; (ii) submicro-methods in which sample sizes below 1000 μg are employed; (iii) the introduction of completely new principles for elemental analysis.

MICROANALYTICAL BALANCES

Before the methods for the determination of the different elements are considered in detail, some remarks must be made on the instrument that is still the most important for quantitative analysis: the balance. The first major improvement in microbalance design was made with the introduction of the air-damping. Microanalytical weighing then became a relatively straightforward process and, more important, the time for one weighing was shortened considerably. However, only with the introduction of the so-called substitution principle, where the balance is always under a constant (maximum) load and so possesses invariably the same sensitivity, has a real breakthrough in balance-making and in weighing technique been achieved. The reading can be taken almost immediately provided that thermal equilibrium after loading the balance has been attained. Still more important, the possibility for weighing errors has been almost eliminated.

With the growing need for methods in which sample sizes below 1 mg are used, the development of 'ultra-microbalances' became a necessity. Today, there is a choice between two different types, the scaled-down beam balances and several brands of quartz torsion/beam balances, with sensitivities between 10^{-7} and 10^{-9} g.

The Mettler Company has introduced two types of ultramicrobalances, one having a maximum capacity of 100 mg with a weighing range of 2 mg and an accuracy of $\pm 0.2 \mu\text{g}$, and the other having a maximum capacity of 500 mg with a weighing range of 10 mg and an accuracy of $\pm 1 \mu\text{g}$. Both balances are top-loaded and have to be used with boats of specially adjusted weight.

The Oertling Company has developed a quartz torsion balance based on the work done by Kirk *et al.*¹ and El-Badry and Wilson² and in cooperation with Belcher and his School³. This balance has a capacity of 250 mg (maximum load on each pan); the weighing range is about 1 mg, the sensitivity $0.02 \mu\text{g}$, and the accuracy $0.05 \mu\text{g}$. The balance uses the torsion-restoration principle. Another quartz torsion balance is the Rodder ultramicrobalance, Model E, with a maximum load capacity of 200 mg and an accuracy of $0.03 \mu\text{g}$.

With the development of instrumental methods for the determination of the final combustion products in recent years, it will be of ever-growing importance to have microbalances where the weight can be transformed into an electrical signal. With reliable balances of this type it will be possible to have fully automated instruments where the percentage of the elements to be determined will be printed out directly. Microbalances of this type with an appropriate maximum capacity and an overall accuracy of $\pm 1 \mu\text{g}$ have not yet been produced, but it can be expected that such balances will become available eventually. Today some 'electrical' balances are on the market; all have the highest accuracy in the lowest weighing range. Two of these balances are standard equipment of two commercially available CHN-analyzers, which will be described below.

MODERNIZATION OF CLASSICAL METHODS

Elemental analysis consists of two stages: in the first stage the organic material is decomposed and the test element is converted into an inorganic compound, whilst in the second stage the amount of the element to be determined is measured. In this review of new developments, the two stages will be considered together wherever possible.

Determination of carbon and hydrogen

The determination of carbon and hydrogen used to be the most time-consuming of all organic elemental methods, as every microanalyst with over 10–15 years of experience in the subject will well remember. With the introduction of the new combustion catalysts developed by Körbl⁴ and Večeřa⁵ (the thermal decomposition products of silver permanganate and tricobalt tetroxide, respectively) the total time per analysis could be shortened considerably. Another advance has been made with the general acceptance of manganese dioxide⁶ as an external absorbent for nitrogen oxides instead of lead dioxide in the combustion tube.

The empty-tube combustion method, developed more than 20 years ago by Belcher and Ingram^{6,7}, has recently become of growing importance in combination with the new instrumental methods. As described by Belcher and Ingram^{6,7}, the combustion is conducted in a dynamic system, but static combustion in a pure oxygen atmosphere seems to be the more generally useful combustion procedure, not only for determinations of carbon and hydrogen but also for the determination of other elements. This principle, which is essentially the same as that used for the determinations of halogen and sulphur, was first proposed by Ingram⁸ for the determination of carbon and hydrogen; a diagram of the apparatus is shown in *Figure 1*. An apparatus based on this type of combustion is now commercially available (A. H. Thomas Company); a rapid

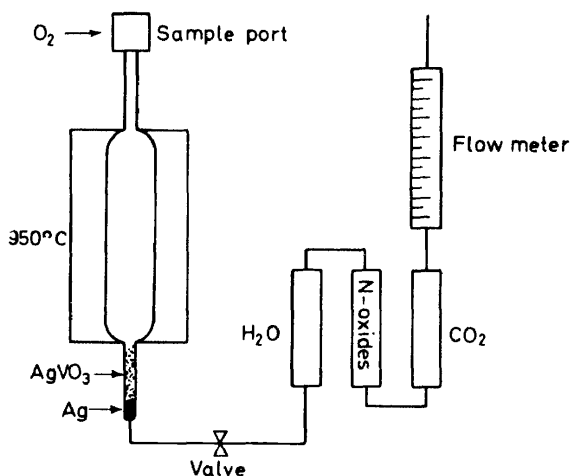


Figure 1. Static combustion method of Ingram⁸

weighing technique for the absorption tubes is employed, so that one determination can be completed within eight minutes. With one of these instruments the number of analyses per day and analyst increased in our laboratories by over 30 per cent. Naturally the gravimetric finish can be replaced by an instrumental final determination.

Based on the work of Marek⁹, the Russian scientists Korshun and Klimova¹⁰ have developed a rapid method which involves pyrolytic decomposition of the test substance in a special container, placed in an empty combustion tube, followed by oxidation of the active decomposition products with an excess of oxygen. The fact that there is no packing in the combustion tube has made it possible for numerous methods for the simultaneous gravimetric determination of several elements in the same sample to be developed by Korshun and her pupils¹¹. It is interesting to note, however, that these methods have not so far achieved widespread application outside their country of origin.

In this connection the findings of Pella¹² should be mentioned. Pella has proved that the carbon and hydrogen contents of organic nitrogen-containing compounds can be determined without the use of any reagent for the absorption of nitrogen oxides, provided that the combustion is conducted in the following way. The sample is instantaneously decomposed in a first static pyrolysis stage in an atmosphere of nitrogen, and in a second dynamic state the products of pyrolysis are oxidized in a stream of oxygen (*Figure 2*). For this reaction Pella suggests the expression 'autoreduction'.

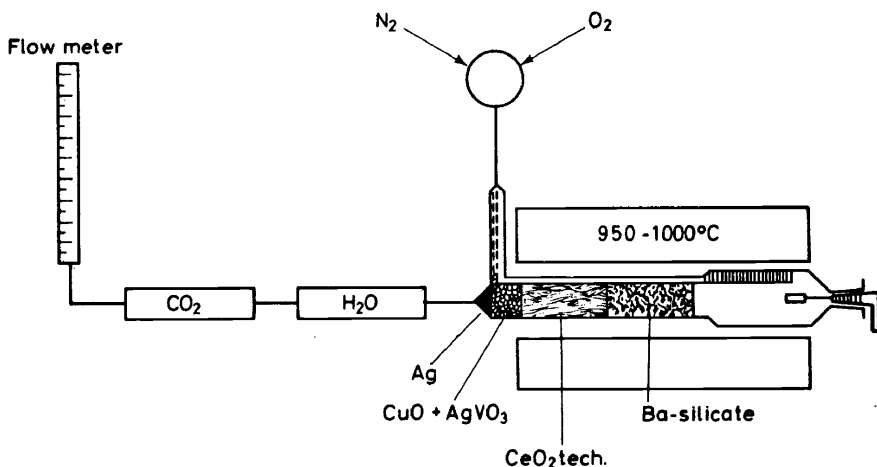


Figure 2. Autoreduction method of Pella¹²

Combustion at temperatures over 1200°C in a rapid stream of oxygen has been proposed by Malissa¹³ and is used with a conductometric finish which will be described later.

For the determination of the combustion products the gravimetric finish is still most widely used. Here the technique of weighing 'on time', which is possible only with the above-mentioned Mettler balances must be mentioned. Soda-asbestos has been used as an absorbent for carbon dioxide since Pregl's

times, but the recent introduction of a specially prepared lithium hydroxide for this purpose offers real advantages. This reagent absorbs 75 per cent of its weight of carbon dioxide, and is very light compared with soda asbestos; moreover, the volume does not increase when absorption takes place.

Trutnovsky^{13a} has proposed a semi-automatic weighing system. The specially designed absorption tubes are mounted in the balance and connected by means of a long Teflon tube to the combustion apparatus. Weighing is done without disconnecting the whole system. The future will show if this 'up-dating' of the gravimetric method of completing the determination is really useful in comparison with the various physicochemical instrumental procedures now available.

Final determinations other than the conventional gravimetric techniques have been published in abundance in recent years, but only those that are past the experimental stage are discussed below. Methods based on the measurement of thermal conductivity of gases, and a manometric method will be described later, when the procedures for the simultaneous determination of carbon, hydrogen and nitrogen are discussed.

Malissa and coworkers¹⁴ have recommended a conductometric method of completing the determination by measuring the decrease in the conductivity of an alkaline solution caused by absorption of carbon dioxide; water can be determined in a similar way after suitable conversion reactions. Salzer¹⁵ has refined this method; carbon dioxide is determined as outlined above, whereas water is determined coulometrically by means of the so-called Keidel cell¹⁶ (Figure 3).

A titrimetric procedure in which a non-aqueous titration is used for carbon dioxide, and water is determined in the same way after conversion to carbon dioxide as described by Unterzaucher¹⁷, is perhaps of greater interest, since this

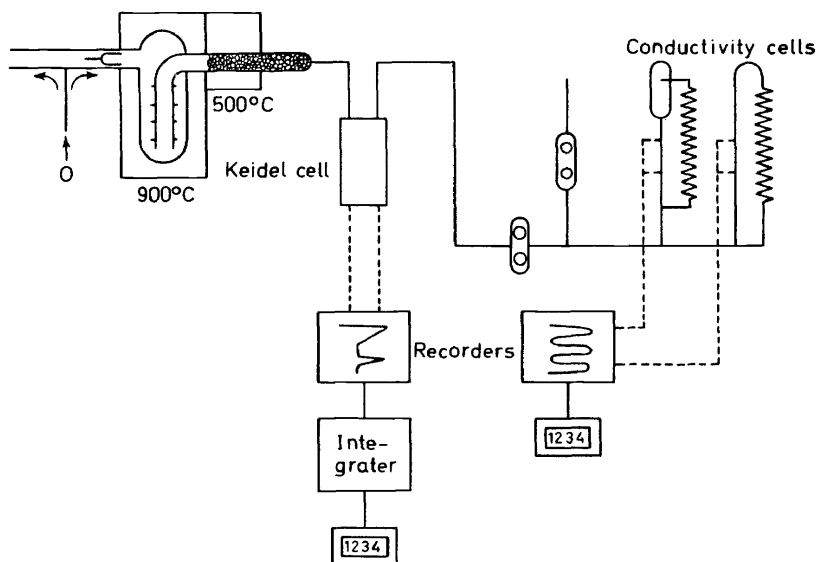


Figure 3. Conductometric-coulometric method of Salzer¹⁵

method can be easily mechanized, as has been shown by Blom and coworkers¹⁸ and by Snoek and Gouverneur¹⁹.

Another approach is the manometric final determination, which became of interest when extensive research with ¹⁴C-labelled compounds started. A mechanized apparatus has been described by Van Leuven and Gouverneur²⁰ (Figure 4); the same principle can be used for a simultaneous determination of carbon, hydrogen and nitrogen.

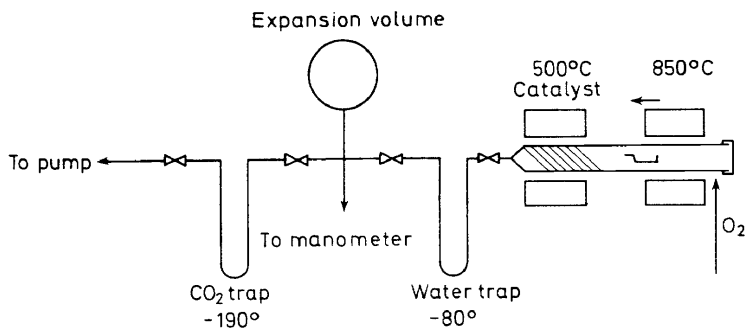


Figure 4. Manometric method of Van Leuven and Gouverneur²⁰

Determination of nitrogen

In the early days of microanalysis, the micro-Dumas method was the determination which needed the most personal attendance. More than 10 years ago the rapid procedures described by Shelberg²¹ and Schöniger²² greatly simplified this analytical problem. Later, Gustin²³ used the same principle to develop a fully automated apparatus, which was made commercially available by the Coleman Company. The most recent development in the field of nitrogen determinations by dry combustion has come from Merz²⁴. In his method (Figure 5), the Ingram combustion system⁸ for carbon and hydrogen is

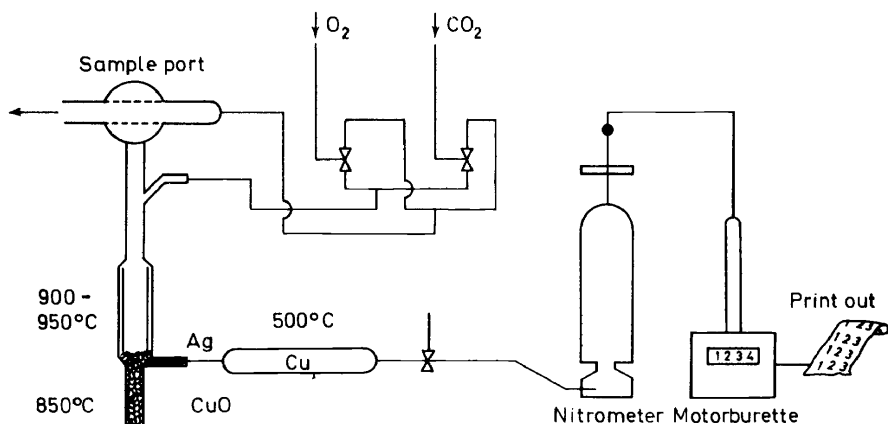
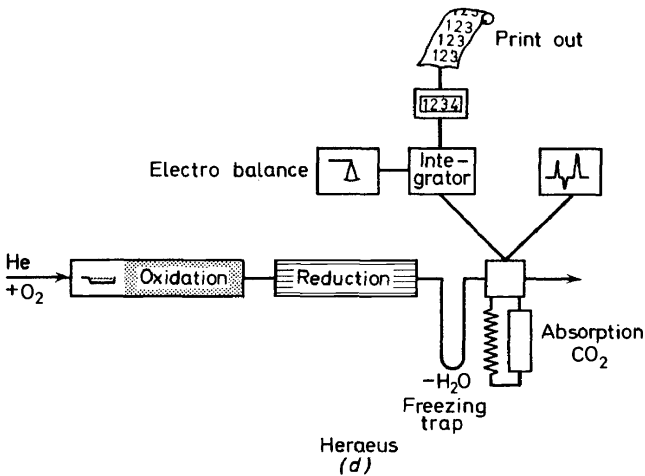
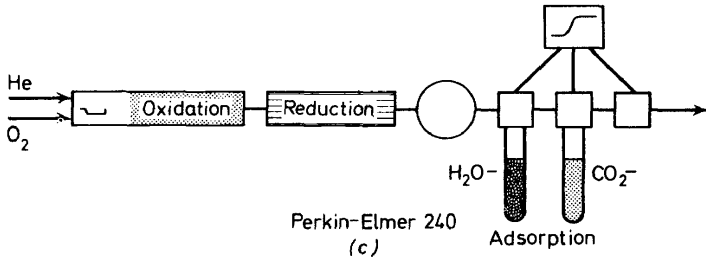
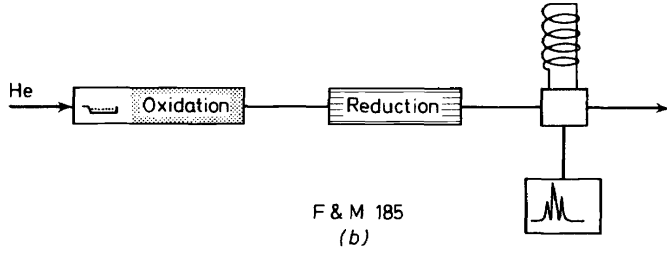
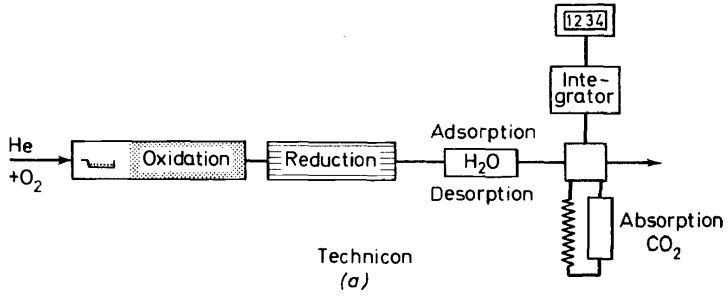
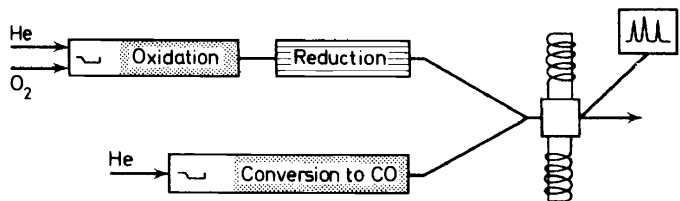
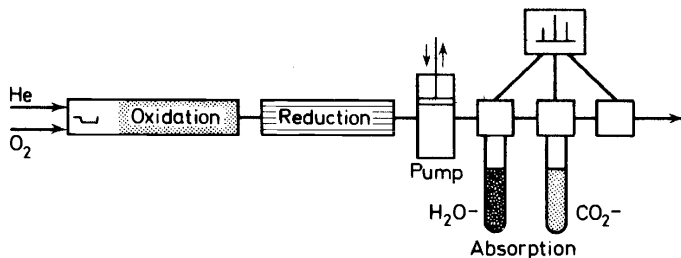


Figure 5. Automatic method for nitrogen (Merz²⁴)

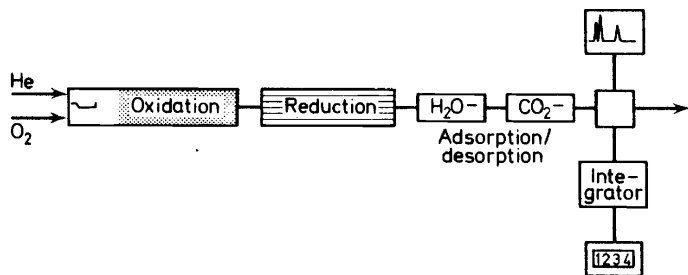




Carlo Erba 1100
(e)



Yanagimoto
(f)



Kainz - Wachberger
(g)

Figure 7. Automatic methods for the simultaneous determination of carbon, hydrogen and nitrogen. (a) Technicon instrument; (b) F and M Model 185 instrument; (c) Perkin Elmer Model 240 instrument; (d) Heraeus instrument; (e) Carlo Erba Model 1100 instrument; (f) Yanagimoto instrument; (g) Method of Kainz and Wachberger³⁵.

employed, the test material being decomposed in a closed atmosphere of oxygen; the combustion products are then swept over copper with a rapid stream of carbon dioxide into a device called an Azotomat—developed first by Monar²⁵—where the nitrogen volume is measured automatically. This apparatus is now commercially available from Heraeus, optionally with a print-out for the determined volume. Naturally, this gas-volumetric method of completing the determination can be replaced by any other method, e.g. by measuring the thermal conductivity of the gases, if suitable carrier gases are selected.

Simultaneous determination of carbon, hydrogen and nitrogen

After gas chromatography had been introduced over 15 years ago as a new analytical method, it was only a matter of time before the principle would be

used for the determination of the combustion products in organic elemental analysis. Actually only five years passed before the first publications on this subject appeared (Walisch²⁶, Simon and coworkers²⁷). The ultimate goal was, right from the beginning, to develop a more or less automated system for the simultaneous determination of carbon, hydrogen and nitrogen. Today, such instruments are commercially available from several instrument firms, and some of these have already proven their usefulness for daily routine work. The points of difference between these instruments are indicated in tabular form in *Figure 6* and are also illustrated in *Figure 7*, which contains diagrammatic representations of the various apparatus. Two other procedures which utilize either thermal conductivity measurement or the above-mentioned manometric finish are also listed. This manometric procedure by Frazer²⁸ seems to be of some interest. The sample to be analyzed (3–5 mg) is weighed into a quartz tube along with 1 g of CuO. This tube is then evacuated, sealed and heated to 925°C. After cooling, the bomb is transferred to the vacuum apparatus and opened and the combustion products are measured. According to the authors, results having an accuracy of ± 0.03 per cent can be expected from this fully automated measuring unit which is controlled by a computer.

To date, too few comparative data obtained by independent investigators with the different commercial instruments are available for a comprehensive judgement to be made. However, experience has shown the following statements to be valid. Despite the fact that all manufacturers claim their instruments to be automated, too much personal attendance is still required, although at least some further manipulations could be readily mechanized. All procedures, where sample amounts below 1 mg are used, have an inherent difficulty from the problem of sample homogeneity. Optimal performance with regard to the quality of the results and to economy can be obtained only when the instruments are used daily. It is not true, as some companies claim, that these instruments can be operated by anybody after a training of only a few hours. On the contrary, not everybody who can be trained for 'classical analysis', is able to analyze properly with these systems. Good maintenance services by the manufacturer should be available because other than simple replacement is beyond the skill of the average laboratory technician. So long as it is borne in mind that none of these instruments will replace all the conventional apparatus, they can be—with the limitations outlined above—very useful.

Determination of oxygen

It is well known that the method for the direct micro-determination of oxygen in organic compounds was developed 'only' 30 years ago by Unterzaucher²⁹ and Zimmermann³⁰. This method is now in general use in larger analytical laboratories. All organically bound oxygen is converted to carbon monoxide and, in principle, this can be determined directly. However, although some chemical methods are known for the direct determination of carbon monoxide, only with the introduction of instrumental methods of completing the determination, such as those described above for simultaneous determinations of carbon, hydrogen and nitrogen, has the quantitative determination of the carbon monoxide itself achieved reasonable application. Several instrumental methods are already known, for example, the methods of

Ehrenberger and Weber³¹, or Dugan and Aluise³². The automatic analyzers for oxygen available from the Heraeus and C. Erba Companies are based on this principle.

However, the carbon monoxide is customarily oxidized to carbon dioxide. In the original Unterzaucher²⁹ method, anhydriodic acid (HI_3O_8) serves as the oxidant and it becomes possible to use an iodometric final determination with increased sensitivity due to the fact that the iodine formed in the reaction can be oxidized to iodate. Very often, copper oxide is used as the oxidant and the carbon dioxide can then be determined by all the known methods described above for the determination of organic carbon. Here, only the nonaqueous titration of Blom¹⁸, the conductometric finish of Salzer³³, the automatic coulometric final determination of Fraisse and Lévy³⁴ and the gas-chromatographic procedure of Kainz and Wachberger³⁵ are mentioned.

Some of the manufacturers of automatic analyzers for carbon, hydrogen and nitrogen claim that their instruments can easily be used as automatic oxygen analyzers, merely by exchanging the combustion train. Although this is of course possible, it will not generally be practicable for routine work. When the need for instrumental determinations of carbon, hydrogen, nitrogen and oxygen exists, the use of two instruments should be regarded as more reasonable; only the automatic analyzer from C. Erba (*Figure 7e*) is an exception.

Determination of hetero elements

Quite often, organic compounds contain, in addition to carbon, hydrogen, nitrogen and oxygen, some other elements, such as halogen(s), sulphur, phosphorus, to mention only the most frequent. What has been said for the determination of carbon and hydrogen is also valid here: in the first step the organically bound element to be determined has to be converted by some means into an inorganic form, and in the second step, the latter has to be determined quantitatively, usually by some method of inorganic analysis.

For the first stage, the mineralization of the organic material, an oxidative or a reductive approach is possible in principle, and very many such techniques have been proposed. However, oxidation techniques are nowadays most widely used. Here, the combustion in a closed atmosphere of oxygen in all its variations from the simple flask combustion to the most elaborate apparatus must be mentioned³⁶. The advantages of this combustion method are found in the following facts: (i) combustion temperatures well over 1000°C are obtained, (ii) the inorganic compound formed can be absorbed in very small amounts of absorption solution thus giving a high ionic concentration of the element to be determined, and (iii) absorption solutions can usually be kept simple so that salt effects on the final determination can be avoided. One of the latest applications of this flask combustion principle is an automatic sample oxidizer from Packard for tritium determinations by liquid scintillation counting, based on work done by Kaartinen³⁷.

Another important oxidation method is the so-called Wickbold³⁸ combustion in all its modifications. Originally developed for the determination of trace amounts, this method is today very widely used, especially for fluorine determinations and for trace analysis, since there is almost no limitation on the

sample size. Extensive studies made by several analysts^{39, 40} have proved the usefulness of this method.

For some particular analyses, it may be advisable to employ oxidation with sodium peroxide in the so-called Parr or Wurzschmitt bomb⁴¹ or wet oxidation with nitric acid and (or) sulphuric acid, the Carius mineralization method. From experience it can be recommended that when completely unknown compounds have to be analyzed, at least two different methods of decomposition should be used to verify the results, especially if only the content of the hetero element has to be determined.

With regard to the reduction methods, no major progress has been made in recent years. Reduction with potassium for the determination of sulphur, as described by Zimmermann⁴², is still used—in some places with magnesium instead of potassium, as has been suggested by Fedoseev and Ivashova⁴³ and Schöniger⁴⁴. Here the work of Jeník⁴⁵ should be mentioned; Jeník recommends this reduction not only for the determination of sulphur and halogens but also for some other elements.

One very old principle, namely catalytic reduction with hydrogen⁴⁶, has been neglected. It could be worthwhile to study this very elegant method thoroughly.

It is beyond the limits of this review to discuss the different possibilities for the determination of the final products of the various decomposition methods in detail. In general, it can be stated that the gravimetric micromethods introduced by Pregl half a century ago are long outdated. There are sufficiently sensitive and selective volumetric methods for every element in question.

For the determination of chloride, mercury(II) nitrate or perchlorate is now very commonly used as a standard solution instead of silver nitrate. The advantage lies in the fact that with mercury(II) ions, no precipitation of the halide is involved so that more sensitive end-points can be achieved with visual indicators. As an indicator, dephenylcarbazide or diphenylcarbazone is used and, following a recommendation from Cheng⁴⁷, the end-point is greatly improved when the titration is carried out in 80 per cent alcoholic solution.

For the determination of bromide, the same procedure can be used, but because of the higher atomic weight of bromine the conversion factor is unfavourable. It is therefore better to use the multiplication titration recommended originally by Kolthoff and Yutzy⁴⁸. Bromide is oxidized with sodium hypochlorite in a buffered solution to bromate. The excess of oxidant is destroyed and after addition of iodide the acidified solution is titrated with thiosulphate.

For the determination of iodide the well-known Leipter procedure is standard practice.

Of the different instrumental methods for the determination of these halides, potentiometric procedures and coulometric methods should be mentioned as being valuable in some cases.

The determination of fluorine in organic substances still causes some problems. Not only is there no general applicable mineralization procedure, but also the problem of the determination of the fluoride ion formed has not yet been completely solved.

Usually titration with thorium nitrate has been used, often as described by Steyermark⁴⁹ some years ago. A more generally practicable method, in which

the fluoride is determined colorimetrically after reaction with lanthanum(III)—alizarin complexan has been described by Belcher and coworkers⁵⁰. It is to be hoped that, especially for the fluoride determination⁵¹, titrations using ion-selective electrodes will shortly become the method of choice.

Sulphur, a very important hetero element, is converted into sulphate ion by means of an oxidative decomposition. For the determination of sulphate, the proposal of Fritz and Yamamura⁵² to use barium perchlorate as the titrant and thorin as the indicator in an 80 per cent alcoholic medium has proved a very great advance. Some other indicators have been suggested, especially by Buděšinsky⁵³. Of the available instrumental methods the coulometric determination of Debal and Lévy⁵⁴ should be mentioned.

When a reductive decomposition method is used, the sulphide formed can either be determined iodometrically, as suggested by Zimmermann⁵⁵, or colorimetrically as methylene blue, the latter being an extremely sensitive method.

Phosphorus, another important hetero element, can be determined either colorimetrically by the molybdenum blue method, or volumetrically as recommended by Püschel and Wittmann⁵⁶; in the latter method, a standard solution of cerium(III) is used with eriochrome black T as the indicator.

To summarize the present situation with regard to the determination of hetero elements, the following points should be made. Titrimetric procedures with an indicator have the advantage that they are faster than methods where the end-point is determined instrumentally. However, instrumental methods can be more easily mechanized, thus providing the possibility of a more or less complete automation. Very recently, a commercial photometric titrator became available, which shows several advantages over earlier instruments for the same purpose.

The progress made in the development of ion-selective electrodes during recent years should have an increasing influence on the final determination of hetero elements in the near future.

SUBMICROMETHODS OF ANALYSIS

In connection with the immense progress in the fields of biology and biochemistry, it has become a necessity to have methods for the determination of elements and functional groups, which can be applied when the sample weight for one analysis is one-hundredth of the standard sample weight for microanalysis. This is exactly the same scaling-down operation as was achieved by Pregl half a century ago in converting macro- to micromethods. The credit is principally due to Belcher³ and his school that we have today submicro methods for all common elemental determinations^{3, 57} and most of the important functional groups. It is impossible to go into details of these very elegant procedures here; details given in at least two monographs^{3, 58}. It should be mentioned, that extensive studies on submicro methods for a sample range of 100–500 μg have been made by Kirsten⁵⁹. In recent years, Tölg⁵⁸ has developed methods for elemental analysis for extremely small sample sizes below 10 μg .

These methods are of great importance for applications when the amount of the sample available for analysis is very limited. They are by no means intended to replace micro methods, which are well established and will remain

the most valuable and convenient for routine analysis. However, some applications will be found also in the field of organic chemistry, when the yield of a synthesis is of the order of 1 per cent or less, or when some side reactions have to be clarified. Another example where submicro analysis can be valuable is for the determination of the amount of hetero elements in substances separated by paper chromatography, as has been shown in several publications⁶⁰⁻⁶³.

NEW PRINCIPLES FOR ORGANIC ELEMENTAL ANALYSIS

It is beyond the scope of this article, where the present status of microanalysis is discussed, to go into detailed speculations about what the near future will bring. However, some new physicochemical methods and their usefulness for elemental analysis should be mentioned briefly.

At first glance, mass spectrometry seems to be excellently suited to elemental analysis; but there are limitations which make a general application nearly impossible. Only a high-resolution spectrograph can be used, so that costs are still extremely high, and, on the experimental side, it is essential to have a molecular peak, which cannot be achieved if the compound to be analyzed decomposes under the given conditions. Moreover, the time for one total analysis is high.

X-ray fluorescence will probably gain in importance in the future. With the apparatus available now, elements with an atomic number higher than 10 can be determined quantitatively, often without previous destruction of the material to be analyzed. However, here again, the economic factor must be taken into consideration. Nevertheless, this method will become of much greater importance in organic analysis, not in the field of elemental analysis in the usual sense, but for the determination of trace amounts. The same applies to atomic absorption spectroscopy, though this excellent method suffers from additional limitations, because it is only applicable when metals—preferably in trace quantities—have to be determined, and in most cases only after destruction of the organic compound.

Finally, some remarks should be made on the possibilities which electronic data processing offers to the larger routine laboratory. Theoretically, a model can be constructed where all the experimental data from the laboratory are fed directly into a computer. Here they are processed, stored and either printed out on the report sheets or directly communicated to the user. Repeat analyses can be ordered directly from the computer, and for unknown compounds molecular formulae or atomic ratios can be proposed. However, the costs of such a system would be out of proportion to the costs of the laboratory itself, and to the benefit obtained. Moreover, such a system would be insufficiently flexible. Certainly, some of the paper work which is connected with every analytical laboratory will be gradually taken over by electronic data processing, but totally automated microanalytical laboratories will never be possible, unless completely new and perfect analytical procedures are discovered.

It can be safely stated that organic microanalysis, as it is conducted today, will prove its usefulness for many years to come.

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