HANDBOOK OF PRACTICAL ORGANIC MICROANALYSIS

Recommended Methods for Determining Elements and Groups

S. BANCE, B.Sc., C. Chem., F.R.C.S.

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CHAPTER 1

Introduction

Since the time, about 1912, that Fritz Pregl introduced micro methods, using 3–5 mg of sample, for the determination of elements such as carbon, hydrogen and nitrogen in organic compounds, the subject (commonly referred to as microanalysis) has seen enormous changes.

The rate of discovery of individual organic substances either by extraction from natural sources or, more often, by synthesis, has increased in an almost explosive fashion.

At first, after Pregl, it was common for the organic chemist to carry out his own microanalysis but it was not long before this work was more often entrusted to a specialist laboratory. Such laboratories found the demand ever increasing and this led to the invention of much more rapid methods and, especially in recent years, to the design of elaborate and costly instruments which will allow a single person to make twenty, thirty or more determinations of carbon, hydrogen and nitrogen per day by almost automatic methods.

Use of such instruments is economically justifiable only where the work load is large enough, and many such laboratories do exist, including some offering contract service, but the present author offers, from his experience of over 30 years of the work, the description of well tried methods (often using very simple procedures and equipment) for all the elements commonly determined.

Carbon, hydrogen and nitrogen are the most frequently determined elements in organic compounds. Consequently C, H & N automatic analysers have proved themselves a great boon to the microanalyst. However, it seems that to make an apparatus which is more rapid it has to be more elaborate and more complicated and consequently more expensive. Not only are they expensive to buy but they cost more to keep in action, for example they usually consume helium and specially pure oxygen all the time. The economics of each situation has to be weighed up. With older methods and no shortage of assistants, carbon, hydrogen and nitrogen can be determined very satisfactorily. The difficulty is often that of obtaining enough assistants possessing sufficient skill and aptitude. In the author's opinion and as a rough guide, as soon as a laboratory finds it has to deal with an average

of 10 samples every working day, each requiring C, H & N determination, then the purchase of a C, H & N analyser is advisable even though it may seem under-utilized since it is easily capable of dealing with more than 20 samples a day.

However, as soon as the work load and number of assistants in the laboratory imply that older methods would be too slow to deal with the demand, then consideration must also be given to providing a second automatic analyser as a reserve should the first temporarily break down. This extra expense must be considered. Where the number of samples is regularly an average of more than 20 a day it is probably justified. Laboratories which use these instruments continuously find they have to have someone (or even several people) with an understanding of them readily available, who can detect and remedy faults in the event of break-down. The instruments cannot be expected, like a washing machine or a television set, just to be switched on and to work without trouble.

In any laboratory there may be one of two possible situations. In some laboratories certain determinations, that of phosphorus, for example, will be needed only occasionally, whereas in others the determination may be carried out all day every day. When the latter is the situation, whatever the determination in question, the laboratory then becomes expert and experienced in the method, and its limitations, advantages and the precision to be expected are well known. On the other hand most laboratories need methods, not only for phosphorus but probably also for metals, water, fluorine, boron and silicon, which, although performed only occasionally, will still give good results without much trouble. The following pages describe many such methods. It is necessary to include the fullest details of the procedure in each case so that the occasional determination can be expected to be successful.

Samples submitted for quantitative analysis may have been extracted from natural sources, or more frequently, have been prepared by synthesis. They will either be solid or liquid at laboratory temperature (gaseous products can also be analysed but are not considered here). From the analyst's point of view, however, there are four classes of sample. There are solids and liquids which can be weighed in open vessels in air without change in weight and there are those which change in weight. These may pick up water, oxygen or carbon dioxide from the air or they may lose weight because they evaporate (or sometimes, unintentionally, they contain a solvent which evaporates continuously). Nowadays, usually in a laboratory where there is a continuous demand for repeated determinations of the same kind, routine work is entrusted to young and only partly experienced assistants. They are asked to follow closely defined directions. Such directions therefore must also be of four kinds, not just for the easiest, that is the solid having a weight that is stable in air, but for solids and liquids, stable and unstable.

A quantitative analysis is usually performed in order to identify the sample material; in the case of a natural product it is the first step in assigning to it a possible molecular formula and in the case of a synthetic product it is usually

simply confirmation of a postulated formula that is hoped for. To obtain the theoretical figures does not, of course, show which of many possible configurations it may be, but not to obtain theoretical figures does show that the sample is not what it was postulated to be.

In addition, quantitative analysis by an experienced laboratory is often used, although this is not the primary purpose, to show how pure, within limits, a given sample of a known substance is. Although the errors are comparatively high (1% relative for most elements but usually less than this for carbon), an analysis that is approximately the theoretical is usually taken as confirming the identity of the substance. It should be noted that given a C, H & N analysis of good precision, a quite approximate halogen or sulphur figure for example will indicate which whole number of these atoms may be present.

At the present time samples can be examined with a variety of physical instruments. This fact sometimes makes the need for quantitative analysis less but usually this (microanalysis) is relatively so easy to do that the information it provides remains well having.

Infrared spectroscopy (IR for short) helps to confirm or detect the presence of certain groups in the molecule. A very useful rapid routine IR service has been provided for many years in the author's laboratory and found extremely useful. Sometimes it tells the synthetic chemist how far a reaction has proceeded and may thus postpone the need for microanalysis.

Nuclear magnetic resonance (NMR) is a technique, especially as applied to hydrogen atoms in organic substances, which is now in very widespread use and is almost an indispensable part of the organic chemists' armoury. It gives information about the kind of carbon and hydrogen groups present in the sample and can be made to give quantitative results. In spite of the existence of this technique, the description of simple *O*-alkyl, *N*-alkyl and acetyl group and also active hydrogen determinations are included in this book because they are relatively easy to carry out and are sometimes needed. In addition they afford practical demonstrations of certain principles of organic chemistry.

The mass spectrometer (MS) can also provide information about a sample. Mass spectrometers vary in resolving power (and cost) but the highest resolution instruments are not indispensable for routine work. The instrument will often provide an indication of the molecular weight of a sample, rendering the older micro methods of molecular weight determination unnecessary.

Gas liquid chromatography (GLC) is an extremely valuable technique, showing among other things whether a sample is pure (i.e. one component only). It is also used to prepare pure samples, usually liquids at room temperature, but should not be regarded as infallible in this.

Thin-layer chromatography (TLC) is also an almost essential modern technique which can be applied to solid compounds in solution.

Solution chromatography has seen a revival recently in the so-called high-performance liquid chromatography (HPLC) where the process is speeded up by

using high pressures to drive a solution of the sample through a column of solid adsorbent. It also can be used either analytically or preparatively.

Thermal analysis in the form of differential thermal analysis (DTA) or differential scanning calorimetry (DSC) can also give valuable information about a sample. Of course, the traditional micro process of observing a sample under the microscope as its temperature is raised or lowered by a heated microscope stage, as in the work of Kofler and Kofler [1], is a form of thermal analysis.

The information obtained by means of all these physical instruments is often extremely valuable but rarely makes unnecessary a quantitative analysis (microanalysis) by the well established methods, which are relatively easy to perform, as described in the pages which follow.

The determination of the amounts of various elements present in organic compounds, the subject of this handbook, is sometimes confused with the other type of microanalysis which can better be called trace analysis. In an absolutely pure organic substance the amount of an element present is substantial, for instance one sulphur atom (a.w. 32) present in a molecule of m.w. of even 1000 would correspond to 3.2% sulphur. Trace analysis on the other hand might be concerned with parts per million concentration levels (1 ppm is 0.0001%). However, it sometimes happens that the microanalyst is asked to determine the percentage of an unintended impurity and although the techniques are not designed for this purpose (the synthetic chemist often does not seem to realize this) the results obtained can sometimes be useful. For instance, chlorine determination by the Carius method (p. 101) can distinguish between 0.2 and 0.3% chlorine or as little as about 0.1% of ash can be measured with reasonable precision (p. 121).

The methods of quantitative organic analysis have developed and improved in rapidity and precision over the years but the most used technique has throughout been that of oxidation of the sample by one means or another without loss of the element to be determined, but with its liberation from organic combination. In other words, the organic compound must be converted into an inorganic species as quickly and easily as possible and then the element of interest can be determined by any method of inorganic chemistry.

Three main techniques using this principle are exemplified in this book.

(1) The first is the complete combustion of the sample by heating it in oxygen in such a way that all the element of interest is converted into a gaseous compound and can be carried in the oxygen stream to an absorber where it is collected. Chapter 4 describes this principle for the determination of carbon and hydrogen by the Belcher and Ingram method.

(2) The second well established method is the combustion of the sample in a closed flask full of oxygen, in which all the products are inorganic in form and retained in the flask. This technique, now so widely used, is described in Section 3.2.

(3) The third method, not now very much used, but simple and effective and therefore extremely useful in some special cases, is the micro-Carius method

(heating the sample in a sealed glass tube with nearly anhydrous nitric acid) and this well tried technique is described in Section 3.1. (The method of destruction of the organic substance by heating in a closed metal bomb with sodium peroxide is retained for only the determination of silicon).

The author has never rigidly adhered to strictly micro quantities of sample (3-5 mg) for determinations and sees no reason why maximum precision should not be striven for by taking a suitable sample size, provided that sufficient sample is available. For example, in the case mentioned above of a sample containing 3.2% S, 10 mg would give a titration of 1 ml of 0.01*M* barium chloride, but 20 mg would be preferable, requiring 2 ml. An even larger sample would be desirable with an ordinary semimicroburette and 0.01*M* barium chloride (which is the traditional 'micro' strength.)

The tendency towards use of semimicro sample sizes makes it generally easier for the young and semiskilled to obtain satisfactory results and removes some of the mystique, that is, the 'this can be done only by an expert' attitude to microanalysis.

During and after the second world war, when most supplies from the continent of Europe were stopped, it was difficult to obtain apparatus for microanalysis. In the hope of improving this situation the British Standards Institution was asked to prepare standards for such apparatus and a whole series was eventually published as parts of BS1428. Such things as weighing vessels, crucibles and filters were standardized, but one of the main goals was to specify sizes so that parts would fit together, for example, in combustion trains. The result is that there exist many specifications, some of which are referred to in this book. Even if an individual does not want the article as specified, the B.S. often provides a convenient starting point for describing the article in question (e.g. the author recommends absorption tubes complying in all respects to the BS except for a slightly larger diameter, p. 40).

It was not until about 1946 that quartz combustion tubing became available. Previously combustion glass, a glass with as high a melting point as was available, was used. Heating was usually by coal gas burners, which in one way was probably just as well because temperatures above 750°C inside the tube were difficult to obtain with gas flames and in any case tubes began to melt slightly and to distort at this temperature. Therefore when fused quartz tubing became available electric heaters were introduced, higher temperatures were possible and consequently combustion was often more rapid and efficient.

BS1428 included a specification of what can be regarded as a standard 5 ml or 10 ml semimicro burette with automatic zero, reservoir and pressure-filling device (BS1428: Part D1: 1965).

In many laboratories these have been replaced by piston burettes (where the rotation of a spiral determines the movement of the piston and thus the volume delivered). The author recommends caution with these, as after a time wear may effect their reliability, and has himself reverted to the more traditional burette

(which can now be obtained in an excellent demountable form, with jet, tap, graduated portion, etc. all joined by ground-glass joints). In any case a typical 5 ml titration must have a better precision than 0.05 ml (1%), represented roughly by one drop of solution.

An experienced microanalyst always keeps certain generalizations in mind. For example, a combustion train needs to reach a kind of equilibrium and therefore is more reliable when in constant rather than intermittent use, and articles such as filter crucibles, which are washed, heated, cooled and weighed are more likely to have reached constant weight if they have been in use a long time. When a new method is tried out and found satisfactory then much time has probably been spent to reach this stage and any suggestion of changing to yet another method is resisted. This produces a kind of conservatism in the microanalyst. The following chapters offer descriptions of methods that have stood the test of time.

If a certain method has to be corrected for a blank which itself has to be determined experimentally, then if it is contrived that the final measurement, often a titration volume, is always of the same magnitude, the absolute value of the blank will be less important, the calculation factor being the most important feature, and this is nearly always determined by carrying out the determination on known pure substances [2] and thus is empirical. If a determination on an unknown substance results in an unexpectedly large or small titration volume, for example, it is better to repeat it with an amount of sample that will give a more suitable titration volume.

It has been found that assistants are very happy to have available in the laboratory the slow but reliable Carius methods for the confirmation of unexplained or unexpected results. Also, even when automatic C, H & N analysers are in constant use it has been found very helpful, almost a necessity, to retain the Belcher and Ingram carbon and hydrogen apparatus for occasional use (p. 35). Among other advantages it will allow the weighing of an ash for example, and will also give the true hydrogen content of a hydrate which might lose water during the sweep step of an automatic analyser.

All methods used may be checked against pure known organic substances. In fact it is true to say that it is better to calibrate all methods against these substances rather than to use theoretical factors. Substances used for this purpose must be easily obtained pure, remain stable indefinitely in air and be non-hygroscopic. They must also be available for all the common elements determined. Such a list was compiled by a committee of the then Society for Analytical Chemistry, was published in *The Analyst* and is available as a reprint [2]. These substances can be obtained commercially in a suitable state of purity.

The following chapters offer selected methods for the determination of all the common elements. Many others may have been tried over the years but those described here have stood the test of time not only for reliability but also for rapidity and ease of performance.