An Automated Digestion Procedure for Food Samples

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This paper was presented to the Annual Conference of the Association of Public Analysts at Taunton, Somerset, on 3 May 1975.

I would like to say something in the context of trace metal analysis about a practical problem which has not received a great deal of attention, namely the automation of sample preparation.

Analysis and Specificity

In a recent lecture to the Society of Chemical Industry[1] on 'Chemical Analysis and Environmental Quality' I set out to describe the role of the analyst in the examination of the environment. Pollution and contamination are matters of opinion, although occasionally the law takes away our discretion in the matter, and *says* for example that, subject to certain defences, it is an offence for most foods to contain more than 2 parts per million of lead. A pollutant can be defined as something which is present in the wrong place at the wrong time in the wrong amount. Analytical chemistry is perhaps the most important tool, which, when coupled with toxicology, gives us the main scientific approach to safety aspects of the environment, and indeed to amenity aspects also. Environment and health are closely related concepts; environmental quality and environmental health are cost-benefit situations.

Most environmental situations which are encountered by the analyst involve trace analysis. The simplest case, perhaps, is fresh water analysis. Although the levels of contaminants may be minute in water, the clean-up problems are often minimal. This is in contrast with the examination of food or other biological material, where the initial removal, by solvent extraction, acid digestion etc., of the great bulk of the organic material, must be accomplished without loss of the trace of contaminant which is to be measured. Indeed, the objective here is to finish up with a simple, clean, aqueous solution of the contaminant, which can then be examined by an appropriate end-method of analysis.

With water, this often is, in fact, the starting point of the analysis. This is true for trace metals such as copper, lead and zinc, which can be measured spectrophotometrically on the water sample itself or after only a simple concentration stage. The advantages and limitations of modern analytical techniques for trace metal pollutants—atomic absorption, atomic fluorescence, atomic emission and mass spectrometry, neutron activation analysis, X-ray fluorescence and anodic stripping voltammetry—have recently been reviewed by Coleman[2]. These methods enable the analyst to deal, in water, with quantities down to about one nanogramme per millilitre (in some cases even less) and of course they do not relate exclusively to the aquatic environment. Selective

ion electrodes are also well established for investigating many inorganic pollutants. A large range is available, the lower limits of detection in water being normally of the order of 0·1 to 0·01 mg/litre, but these electrodes are subject to interference by other ions (the nitrate electrode is a good example) and electrodes should be regarded as 'ion selective' rather than 'ion specific'. The possibility of extending their use to organic compounds by means of immobilised enzyme systems has also been discussed. Such systems cannot, however, be more selective than the enzyme systems they involve.

Specificity is not always a dominant consideration for the trace analyst, but it must be taken into account. This is particularly true for organic pollutants. Where the nature of the contaminant is not known in advance, the specificity of methods of examination cannot be taken for granted. A classic example was the occasion when aldrin appeared to be present in soil samples which had been kept in sealed jars since before 1940, i.e., before the advent of chlorinated insecticides[3]. Gas chromatographic responses, which 23 years later could be properly interpreted, thus subsequently were regarded as being due to naturally occurring interferences.

The situation has been dramatically if somewhat theoretically described by Widmark, in what he has called the *tracer cosmos*[4]. By this he means the vast increase in the number of different trace compounds which become theoretically possible as one passes into the higher realms of analytical sensitivity. As an example, consider a substrate which is 99 per cent. pure. In the simplest case, the impurity is 1 per cent. of one other substance. Alternatively, however, there could be 0·1 per cent. each of 10 different impurities, or 10,000 different compounds each at the 1 part per million level. A number of analyses today are of interest at the parts per thousand million level, or even lower. If all the impurities were present at this kind of level, one might theoretically have to contend with a million or more different trace compounds. The *cosmos* is represented by the integration of all these levels, and they might be represented simultaneously in a 'real life' situation. The disposal of toxic wastes to land —a subject dealt with by the Royal Commission on the Environment in its Second Report[5]—perhaps conjures up an extreme example of the possibilities.

Man is, of course, exposed to the atmospheric environment, to the aquatic environment and the terrestrial environment—less to the second than the first. As far as trace metals and pesticides are concerned, for the greater part of the population, the main exposure is by way of the terrestrial environment—particularly food. Thus, as regards the intake of lead, it has been estimated that some 85 per cent. of the total intake is by way of food, with perhaps only 10 per cent. from water and 5 per cent. from the air[6]. Indeed, Galley has shown from pharmacodynamic considerations and from the minute levels of organochlorine pesticide compounds present in the atmosphere, that man exhales more dieldrin, derived from the trace amounts in his body fat, than he inhales[7]! As far as trace pesticide residue analyses are concerned, the developments in chromatographic techniques (initially paper chromatography, then gas-liquid chromatography and more recently high performance liquid

chromatography) have given the analyst an order of specificity and sensitivity which a generation ago was undreamed of.

The possibilities of interference in these analytical methods are as important as with other methods. Sample extraction and clean-up (often themselves chromatographic in character) must be properly conducted, and due regard must be paid to the need for confirmatory procedures where the identity of a trace contaminant is unknown. Similarly with trace metals; atomic absorption spectroscopy (coupled where necessary with chelation techniques and solvent extraction methods of separation and concentration) has provided the analyst with a powerfully discriminating and sensitive tool. Atomic spectroscopy methods are applicable to some sixty different elements. The limits of detection vary from about 10 microgrammes to about one-tenth of a nanogramme per litre (depending on the element), with flame or high frequency plasma emission sources being somewhat more sensitive than atomic or fluorescence flame sources[2]. These methods can be highly specific, but due regard must be paid to matrix and other possible interference effects. Standard reference materials are highly desirable but are not always available.

In these days of intense interest in the environment, more and more detailed information is sought on the occurrence and distribution of contaminants, and there is one constraint which, even if financial resources were not a consideration, would limit the analyst's practical ability to undertake comprehensive environmental surveys. That is the *preparation* of the sample, for its eventual examination by chromatography, spectrometry, or whatever other end-method of analysis is to be used.

Automated Analysis

There is already a wide range of automatic analytical equipment, much of it available in the pioneering field of clinical chemistry. Many instrumental systems are discrete in character, in that they relate to individual, unit analytical processes. There is, for example, a large range of commercial sampling equipment and colorimetric analysis equipment, with or without sample separation processing facilities. There are a few continuous analyser systems, the main one being the Technicon AutoAnalyzer. Details of all of these have been published by Foreman and Stockwell[8].

A great deal of effort has recently been devoted to the development of instrumental methods for the direct examination of a wide variety of samples. This has not been the situation, however, with the preparatory aspects of the analysis of complex samples of biological origin, such as food or body tissues. Techniques such as X-ray fluorescence spectrometry can look directly at such samples after they have been freeze-dried, but they have limited sensitivity and are unsuitable for trace analyses. Activation analysis has been used in this area but it cannot cover the full range of elements required, without recourse to chemical separations. Most other techniques require some sample manipulation, usually wet or dry oxidation.

In only a few areas do the practical aspects of the work of the Laboratory of the Government Chemist call for the examination of very large numbers of routine samples. Where these do occur, and in certain other cases where a heavy staffing involvement can thereby be reduced, a study has been made of the automation of the analytical methods concerned. Thus the Laboratory has studied the automatic dosing and monitoring of fluoride in water supplies, and the automation of methods for the extraction, identification and estimation of quinazarin[9] and furfural[10] which are the revenue markers in hydrocarbon fuel oils. Analytical processes based on the estimation of alcohol and reducing sugars[11–13] can be used to assess the original gravity of beers. Specificity does not feature strongly in the latter system, which is used mainly as a screening test. Thus the process for the estimation of original gravity is used only to select the occasional sample, perhaps one in 20, which needs to be examined by the full distillation and gravity procedures. The starting point for the automated methods may be commercial equipment, where suitable items are available; but these are usually modified, often profoundly, and occasionally in co-operation with the manufacturers. Usually, however, it has been necessary to build new, special units.

Because of the practical importance of the preparatory stages in routine survey work, the Laboratory of the Government Chemist has in recent years made a special study of how the 'real life' problem of preparation of samples for food analysis might be automated.

The automation of food sample preparation for the determination of toxic substances (which should be suitable also for pesticide residues analysis) by means of grinding, solvent extraction and centrifugal separation, has recently been described by Coulson[14] and is based on the centrifugal separation of Vallis[15] and the principles developed by Anderson[16].

The automatic preparation and wet digestion of biological samples has been neglected, for the very simple reason that it is extremely difficult. It is necessary to manipulate corrosive reagents at relatively high temperatures, and to transfer awkward samples such as food slurries. However, as a result of several years' work, an automatic digestion system has been developed, which combines the main advantages of the Technicon continuous approach, together with some aspects of the system used by other manufacturers. It is currently being tested for a wide range of food samples and is being compared with manual methods of digestion both for reliability and accuracy. The complete system for the digestion of foods for trace metals examination has due regard for specificity, and it comprises a sample introduction unit, a continuous digester, a digest collection and neutralisation unit, a chelation extraction and phase separation unit, and a chelated extract collection unit. It is illustrated diagrammatically in Fig. 1.

The sequence of operations is as follows. A 5–10 grammes sample is weighed into a plastic bag, and a measured amount of 50 per cent. of sulphuric acid is added. The mixture is heated to 60°C in an oven and homogenised in a 'stomacher'[17]. The mixture is then weighed, and a weighed sub-sample of

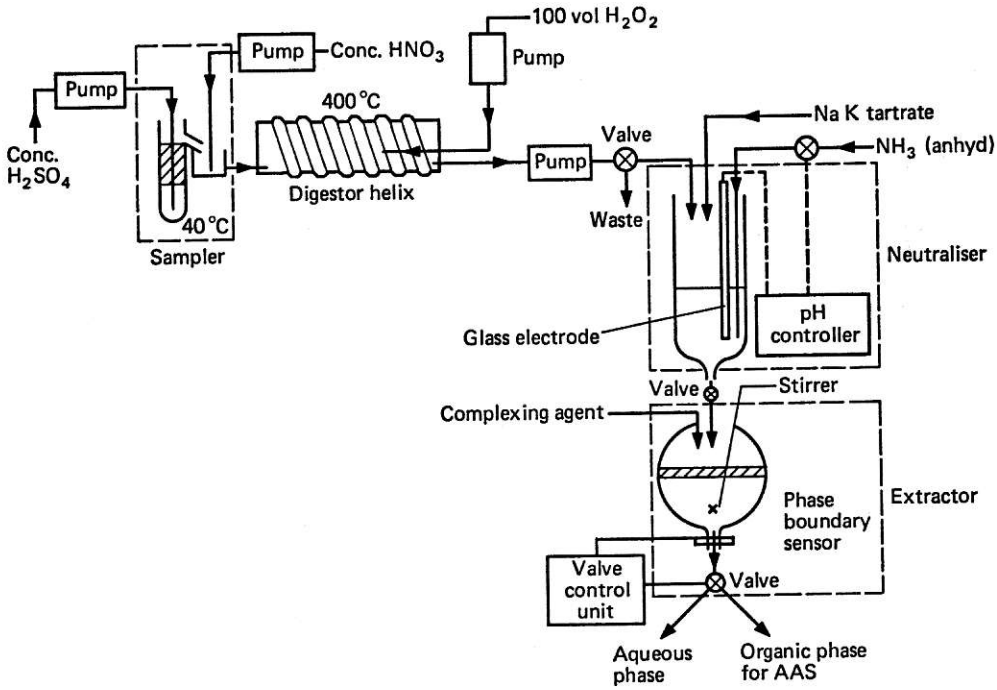

FIG. 1. Automatic Digester System: Flow diagram.

slurry is poured into a glass sampler tube and placed on a turn-table. The processes which follow are completely automatic. A platinum-iridium probe dips into the sample and pumps concentrated sulphuric acid into the bottom of the sampler tube, thus displacing the sample through a side-arm into a rotating helical digester, together with a stream of concentrated nitric acid. Hydrogen peroxide may also be added about half way through the digest process. The sample is digested in the modified Technicon digester, and the strong acid digest resulting from this process is pumped into a water-cooled neutraliser vessel where a glass electrode is used to control a flow of neutralising ammonia gas, in order to bring the final digest to a predetermined pH. The digest is then transferred to an extractor vessel, where a chelating agent is added, and the metals are extracted into a suitable organic solvent. The vessel is emptied through a valve which is controlled by a phase boundary detector so that the aqueous phase goes to waste and the organic phase is retained for analysis, by atomic absorption spectrometry for example.

The modification of the Technicon digester unit, which was necessary to improve the temperature control, has been described by Jackson, Morley and Porter[18], and the phase separation unit, which incorporates a sensor based on change in refractive index, has been described by Porter, Jackson and Bunting[19]. Although the system sounds very simple, a number of important problems had to be overcome during its development. Thus, it was necessary not only

to modify the normal temperature control system of the Technicon digester to give stable high temperatures all the way along the helix but it was also necessary to retain the samples in the turn-table at about 60°C to prevent a separation of fats from the slurry. Careful attention had to be given to the materials of construction. It is necessary to ensure that these can withstand the corrosive action of strong acid and that they do not introduce unacceptably high blanks into the system. Some foods do not break down adequately in the sulphuric acid/nitric acid medium. It is for these that hydrogen peroxide is added about half-way along the digester, thus enabling the subsequent chelation stage for the separation of trace elements from the sample to be achieved more efficiently.

A wide variety of chelating agents was studied in a search for one which extracted the maximum number of elements with virtually 100 per cent. recovery. The most satisfactory was diethylammonium diethylcarbodithioate (DDDC), at pH 3, with heptan-2-one as the extracting solvent. Good recoveries of iron, cobalt, nickel, copper, zinc, cadmium and lead are possible from foods as different as flour and corned beef. The normal ammonium pyrrolidine dithiocarbamate (APDC)[20] chelation system was not found to be suitable for automatic analysis, since the excess nitric acid used in the digestion decomposes the APDC and it is not convenient to boil it off. Moreover, the APDC-metal chelates are less stable. This is a matter of little importance when spectrophotometric measurements are made immediately after extraction, but with an automatic digestion system working at three samples per hour, an expensive on-line measuring system may not be justified if the metal-chelate extracts can instead be collected, stored (perhaps overnight), and examined as a continuous batch. In the search for alternative chelating agents, oxine[21] gave good results, but DDDC was even better. Heptan-2-one is used as an extraction solvent in preference to 4-methylpentan-2-one (MIBK), since the former gives a larger difference in refractive index, and the phase boundary detection system[22] for separation is based on this.

The investigation of a wider range of foods and elements is under consideration, using a sequential chelation system. The process is described in the 1974 Report of the Government Chemist[23].

The digestion unit system which has been described can process about 20 samples per day, and by the combination of units it should become possible to examine a much larger number of samples than has been possible hitherto. This should provide an overall improvement in 'job satisfaction' for the laboratory staff concerned. This also means that it should become possible to increase the number of samples examined and to design trace metal studies in a way which will give ample coverage without their being restricted by staff limitations.

I am grateful to Dr Peter B. Stockwell for discussions on this paper. He and members of his team in the Laboratory have carried out much of the original work upon which the foregoing description is based.

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