The Analyst

The Analytical Journal of The Chemical Society

A monthly international publication dealing with all branches of analytical chemistry

Volume 104 No 1273 Pages 273–384 April 1979

THE ANALYST

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Editorial: The Director of Publications, The Chemical Society, Burlington House, London, W1V 0BN. Telephone 01-734 9864. Telex No. 268001

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Volume 104 No 1237 April 1979

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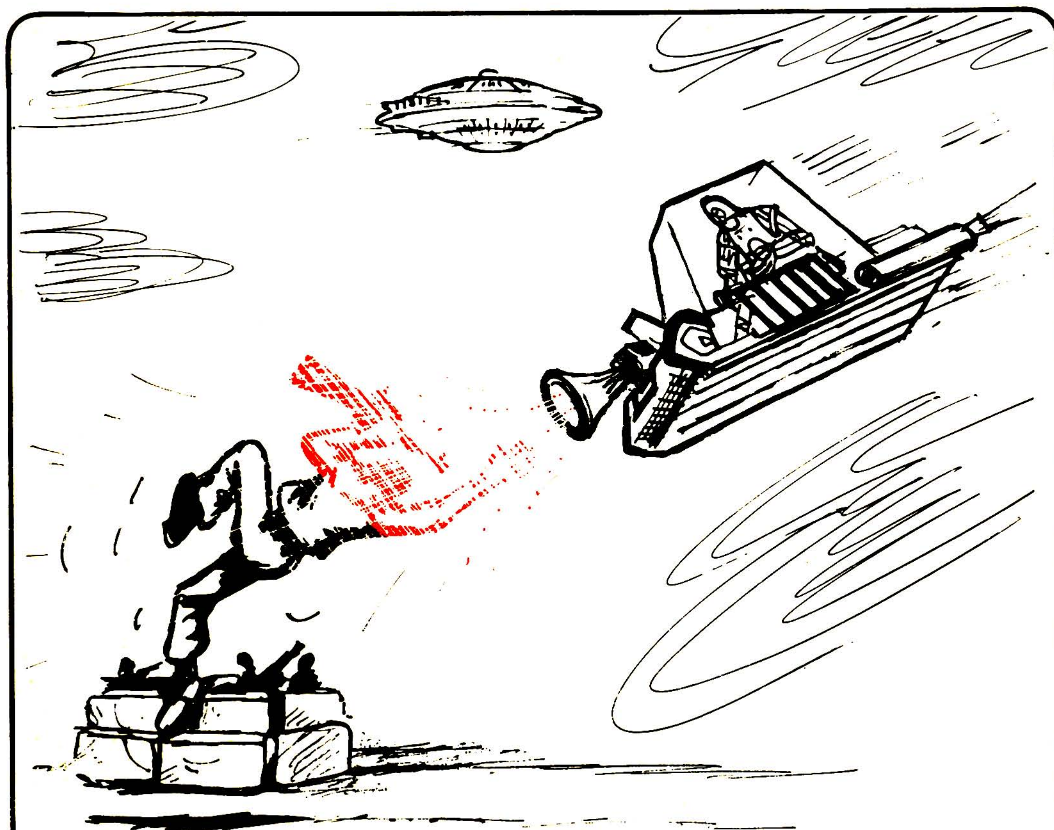

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Summaries of Papers in this Issue

Approach for Achieving Comparable Analytical Results from a Number of Laboratories

In the field of water analysis, growing importance is being attached to the ability to compare, with confidence, the results from different laboratories. However, the errors in the results can invalidate such comparisons. One approach to ensuring results of adequate accuracy is described in this paper. Examples of its successful application to river-water analysis within the UK will be presented in subsequent papers.

Keywords: *Water analysis; accuracy of results; inter-laboratory comparability; analytical quality control*

A. L. WILSON

Water Research Centre, Henley Road, Medmenham, Marlow, Buckinghamshire, SL7 2HD.

Analyst, 1979, **104**, 273–289.

Accuracy of Determination of Chloride in River Waters: Analytical Quality Control in the Harmonised Monitoring Scheme

The Department of the Environment, in collaboration with the Regional Water Authorities, has initiated a Scheme for the Harmonised Monitoring of the Quality of Inland Fresh Waters in England and Wales. The Scottish Development Department has introduced a similar scheme in Scotland in collaboration with the Scottish River Purification Boards. To achieve the required comparability of results from all laboratories involved, each laboratory takes part in an analytical quality control (AQC) scheme; this work is co-ordinated by the Water Research Centre. The general approach adopted to AQC has been described, and this paper presents the tests made and results obtained in the determination of chloride in river waters. Broadly, each of the ten participating laboratories achieved total errors not greater than $\pm 20\%$ of the chloride concentration for concentrations greater than 5 mg l^{-1} of chloride.

Keywords: *River-water analysis; chloride determination; accuracy of results; inter-laboratory comparability; analytical quality control*

ANALYTICAL QUALITY CONTROL (HARMONISED MONITORING) COMMITTEE

Water Research Centre, Henley Road, Medmenham, Marlow, Buckinghamshire, SL7 2HD.

Analyst, 1979, **104**, 290–298.

Statistical Appraisal of Interference Effects in the Determination of Trace Elements by Atomic-absorption Spectrophotometry in Applied Geochemistry

Interference effects in the determination of trace elements by atomic-absorption spectrophotometry in applied geochemistry have been studied by a statistical appraisal and described in terms of a simple two-parameter mathematical model. The experimental design incorporated features that would allow possible deviations from the model to be detected, but no serious deviations were detected except in a deliberately chosen example. The study led to the identification of some important interference effects that could affect interpretation of geochemical data and also provided a formula that could be applied to correct the crude results.

Keywords: *Applied geochemistry; mineral exploration; atomic-absorption spectrophotometry; interferences; chemometrics*

MICHAEL THOMPSON, STEPHEN J. WALTON and SHIRLEY J. WOOD

Applied Geochemistry Research Group, Department of Geology, Imperial College, London, SW7 2BP.

Analyst, 1979, **104**, 299–312.

Minimum Sample Preparation for the Determination of Ten Elements in Pig Faeces and Feeds by Atomic-absorption Spectrophotometry and a Spectrophotometric Procedure for Total Phosphorus

Studies of mineral metabolism in pigs and problems of manure disposal or utilisation are complicated by interactions of trace metals and major cations. A procedure for the determination of copper, zinc, cadmium, lead, iron, sodium, potassium, magnesium, calcium, phosphorus and arsenic in pig faeces and feeds is described. Phosphorus is determined spectrophotometrically and the other elements by atomic-absorption spectrophotometry. Sample preparation is minimised, and all elements except arsenic are determined after a single sample digestion in nitric acid - perchloric acid mixture. A separate sample digestion is necessary for arsenic. The accuracy and precision of the method were rigorously tested, and are suitable for budget studies of all eleven elements.

Keywords: *Pig faeces and feed analysis*; *trace metal determination*; *major elements determination*; *atomic-absorption spectrophotometry*; *spectrophotometry*

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Analyst, 1979, **104**, 313–322.

Sulphochlorophenol N as a Spectrophotometric Reagent for Vanadium(V)

A spectrophotometric method for the determination of trace amounts of vanadium(V) with sulphochlorophenol N is described. With this reagent, vanadium forms a blue complex, which is stable in the pH range 3.7–6.0. The coloured complex obeys Beer's law at 627 nm in aqueous solution with a molar absorptivity of 3.12×10^4 l mol^{-1} cm^{-1}. Copper and cobalt ions interfere in this method.

Keywords: *Bisazochromotropic acid dye*; *spectrophotometry*; *sulphochlorophenol N*; *vanadium determination*

M. ZENKI

Department of Chemistry, Okayama College of Science, 1-1, Ridai-cho, Okayama-shi, 700, Japan.

Analyst, 1979, **104**, 323–327.

Oxidative Determination of Dextromoramide (Palfium) in Body Fluids

The oxidation of dextromoramide to benzophenone with alkaline potassium permanganate and measurement of its ultraviolet absorbance is advocated for the determination of this drug in urine and serum over the concentration range 5–40 μg ml^{-1}.

Keywords: *Dextromoramide determination*; *urine*; *serum*; *plasma*; *ultraviolet spectrophotometry*

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Analyst, 1979, **104**, 328–333.

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APRIL 1979 Vol. 104 No. 1237

The Analyst

Approach for Achieving Comparable Analytical Results from a Number of Laboratories

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In the field of water analysis, growing importance is being attached to the ability to compare, with confidence, the results from different laboratories. However, the errors in the results can invalidate such comparisons. One approach to ensuring results of adequate accuracy is described in this paper. Examples of its successful application to river-water analysis within the UK will be presented in subsequent papers.

Keywords: *Water analysis; accuracy of results; inter-laboratory comparability; analytical quality control*

1 Introduction

Increasing importance is being attached to the measurement and control of the quality of many types of water. This, in turn, has led to a growing need to control the accuracy* of analytical results so that valid conclusions can be drawn when results are compared either with each other or with water-quality standards. Such control generally presents problems because of the many factors that can adversely affect analytical accuracy. In the author's experience, a critical and well co-ordinated approach to such problems is essential, if there is to be any chance of reliable achievement of a specified accuracy in routine analysis. The purpose of this paper is, therefore, two-fold: (*i*) to describe the approach that is being used within the UK to ensure adequately accurate results for the Harmonised Monitoring of River Water Quality[3]; and (*ii*) to introduce a series of papers on the application of the approach to, and the results obtained for, a series of determinands† within that scheme.

Hence, this paper is concerned with a topic (analytical quality control) about which much has already been written. However, to the author's knowledge, there has been little or no integrated discussion of all aspects of the topic relevant to water analysis, although other publications[5–12] deal with one or more of the important aspects. It is hoped, therefore, that the present account of a complete analytical quality control scheme will be of general interest.

The scheme described below was evolved about 15 years ago for a survey of feed-water quality in power stations of the Central Electricity Generating Board. The successful applications to river and other waters suggest that the approach may be of general value. It is interesting, therefore, to note that the scheme is very similar in fundamental concepts and practical aspects to an excellent and authoritative approach recently recommended for clinical chemistry.[1]

2 Approach to Analytical Quality Control

2.1 General Concepts

The approach consists, for each determinand, of sequential completion of a number of individual but closely linked stages as summarised in Fig. 1. These activities and their sequence have been chosen so that generally important sources of error are eliminated progressively or controlled at adequately small values. In this way, a permanently sound

* Accuracy is used here with the sense of "total error" (*i.e.*, the sum of random and systematic errors); accuracy is said to improve as the total error decreases. The total error of a result is the difference between the result and the "true value," it being assumed here that a true value exists for every sample.[1,2]

† Determinand is used here with the sense[4] "that which is to be determined."

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basis for control is ensured with a minimum chance of wasted effort. It is important, therefore, that no stage is started until the preceding stage has been completed satisfactorily. The reasons for, and activities involved in, the individual stages are described in the following sections.

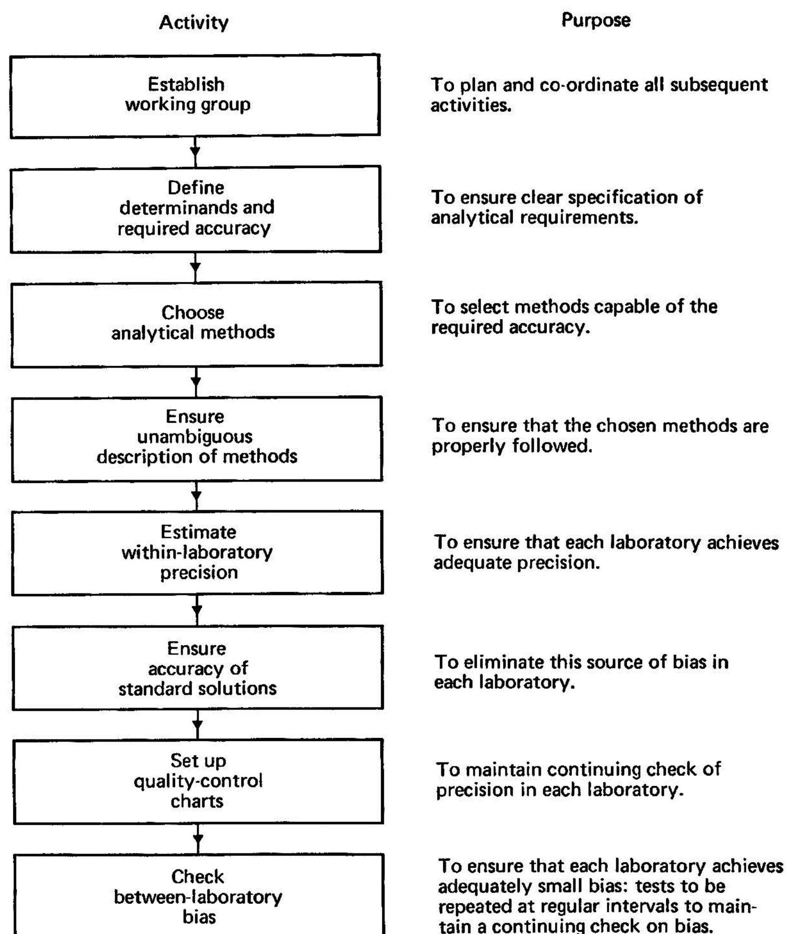

Fig. 1. Sequence of activities for analytical quality control.

The detailed considerations and work necessary in each stage are governed by the particular determinand and the required accuracy. The aim here, therefore, has been to stress the principles involved; subsequent papers will give the information relevant to particular applications. Emphasis has also been placed on analytical aspects and no attempt has been made to explain and give full computational details of all the statistical tests involved. Each such test is, however, stated, and details can be obtained either from the references quoted or from general statistical texts. It is also worth stating that many of the points made below are not novel. Nevertheless, their inclusion is considered necessary because they are sometimes overlooked and, more importantly, they should be seen in the context of an integrated approach to analytical quality control. In particular, as the literature has dealt with the early stages of Fig. 1 to a smaller extent than the latter, the former are treated here in greater detail, especially as they form the basis of all subsequent experimental work.

One aspect of the scheme in Fig. 1 should be stressed at the outset, *i.e.*, everything possible

is done to eliminate sources of error before making tests of between-laboratory bias. There are two main reasons for this. Firstly, it is often difficult in water analysis to obtain a direct, experimental estimate of this bias for all of the many types of sample usually analysed by laboratories. Secondly, if such a bias is found, considerable difficulty is often experienced in eliminating it, particularly when the laboratories are widely dispersed geographically.

2.2 Establishment of a Working Group

To ensure an efficient and uniform approach in all laboratories throughout the stages of Fig. 1, thorough co-ordination of all the work is necessary. At least one laboratory (see below) with the effort and resources necessary to provide advice and to plan and co-ordinate the work is essential; this laboratory is referred to as the co-ordinating laboratory.

The effectiveness of any control scheme rests ultimately on the competence of individual laboratories. Hence, the maximum possible understanding of all aspects of the work should be sought in all laboratories. This point is of special importance because the statistical procedures required in estimating errors may sometimes be unfamiliar to analysts. Understanding and efficiency tend to be improved when laboratories are parties to decisions rather than having particular approaches imposed on them. For these and other reasons, it is of value to establish a Working Group to plan and agree both the general approach and also the detailed procedures for each determinand. Whenever possible, this Group should be composed of a representative from each of the participating and co-ordinating laboratories; other interested organisations may, of course, also be represented. In addition to the particular purposes mentioned above, such Working Groups also act as a useful means of exchange of analytical information.

The number of laboratories and/or their geographical distribution may sometimes be so large that it is impracticable for one co-ordinating laboratory to deal with all laboratories. When this is so, a hierarchical scheme is useful, *i.e.*, the laboratories are divided into a number of groups each of which can be adequately serviced by a co-ordinating laboratory. All the co-ordinating laboratories then form one level in the hierarchy and they follow the scheme in Fig. 1. On satisfactory completion of all stages of the work for a particular determinand, each co-ordinating laboratory then initiates an identical approach within its own group of laboratories.

2.3 Definition of Determinands and Required Accuracy

Unambiguous definition of the determinands of interest[8,11] and numerical definition of the accuracy required of the analytical results are essential for three main purposes in any control scheme: (*i*) to allow the choice of analytical methods appropriate to the intended uses of the results; (*ii*) to provide clear criteria for deciding whether or not the errors observed in particular laboratories warrant corrective action; and (*iii*) to ensure that adequate numbers of tests are made to allow errors of the magnitudes of interest to be detected as statistically significant.

The first task of the Working Group is to ensure that these definitions of the requirements are available because they will form the basis of almost all subsequent work. Much could be written on their formulation, but it suffices here to mention that they often appear to be accorded little or no attention, and to summarise certain generally important points in sections 2.3.1–2.3.6.

2.3.1 *Responsibility for definition of required determinands and accuracy*

Point (*i*) above implies that the requirements should be defined by those who will use the results rather than by those who provide them. In principle, this is correct, but in practice, of course, joint discussions are generally essential.[8]

2.3.2 *Definition of determinands*

Many of the substances of interest in water analysis can exist in a variety of chemical and physical forms, to each of which a given analytical method often responds to different extents. Examples of this and related points are discussed elsewhere.[8,11] It follows that an unambiguous definition of determinands is essential so that appropriate analytical methods can be selected.

Many determinands in water analysis are non-specific,[13] *i.e.*, they do not correspond to one or more particular chemical species (chloride, iron, etc.), but rather express certain properties of samples, *e.g.*, colour, biochemical oxygen demand, which are governed by undefined species. In general, the true values for such determinands in any sample are defined by the analytical methods used for their measurement. Therefore, the determinands can be defined unambiguously only by defining a particular method to be used (see also section 2.4). The same approach may also be necessary for certain determinands that cannot be measured analytically by available methods, *e.g.*, "dissolved" iron. In this instance, as a complete separation of dissolved and undissolved forms is not generally possible, the procedures used for this separation can have markedly different efficiencies. Hence, the separation procedure (*e.g.*, the filtration system) may need to be specified as a means of defining the determinand.

2.3.3 *Sources of error*

Inaccurate results can be caused by errors occurring during sampling (*e.g.*, contamination), between sampling and analysis (*e.g.*, through instability of the determinand) and during analysis.* In water analysis, the first two sources can be very important for many determinands if appropriate precautions are not taken. Clearly, attention must be paid to such errors in addition to those occurring during analysis. However, control of the errors before analysis commonly involves different factors than those relevant to analysis. Further, analysts are often not directly involved with sampling. For these reasons, the author favours two separate but closely linked approaches to the control of sampling and analytical errors and this is used in the Harmonised Monitoring Scheme. A similar suggestion has been made in the context of quality control in clinical chemistry.[1] However, procedures suitable for ensuring constancy of the determinand concentrations between sampling and analysis (*e.g.*, the addition of a chemical preserving reagent) can affect the performance of analytical methods; close co-ordination of these aspects is, therefore, essential. On the above basis, the remainder of this paper is concerned primarily with the control of errors arising during analysis. A subsequent paper will consider the other sources of error.

2.3.4 *Method of expressing the required accuracy*

Finally, there is the question of how to express the required accuracy. This can be done in a number of ways and each scheme should choose the most appropriate for its purposes. However, the approach described below is thought to be of general value in environmental monitoring and in similar applications where the determinand concentrations may cover a relatively wide range.

For each determinand, the smallest concentration† of interest is decided, and this is equated to the limit of detection, L, required of the analytical method. The limit of detection is defined statistically[14,15] with the assumption that the random errors of analytical results follow a normal distribution. The maximum tolerable total error of an analytical result for any sample is then defined numerically by a statement in the form "The maximum tolerable total error of a result is p% of the concentration of the determinand or L mg l^{-1}, whichever is the greater." This method of expression recognises that relative errors increase markedly as the concentration of a determinand approaches the limit of detection. Appropriate numerical values of p and L are chosen for each determinand and scheme. For the Harmonised Monitoring Scheme, p is 20 for most, but not all, determinands; the value of L varies with the determinand.[3,11] Of course, simpler expressions are possible when only a narrow range of determinand concentrations occurs or is of interest.

2.3.5 *Definition of tolerable random and systematic errors*

It is also important to define separately the random and systematic errors that can be tolerated, because they have different effects on the validity of results and because they are estimated in different ways. For the Harmonised Monitoring Scheme, the random error

* Errors arising through clerical errors and/or through the use of different units in reporting results, though important, are not considered here.

† Certain determinands are not expressed in concentration units. For simplicity here, the term concentration is used throughout.

of a result is taken as twice the standard deviation of individual results and the maximum tolerable total error is divided equally between random and systematic errors. This division of total error between random and systematic errors is arbitrary, but experience suggests it is often useful. Other divisions may be used if desired, but experimental problems in checking bias can arise as the ratio of the tolerable random and systematic errors is increased (see section 2.9.3). Thus, numerical values for the maximum tolerable standard deviation and bias of analytical results can be deduced for any concentration of determinand. For example, the maximum tolerable standard deviation is $0.25p\%$ of the concentration of the determinand or $0.25L$, whichever is the greater. It is of interest to note that recent German proposals[9] on monitoring drinking water have recommended maximum tolerable standard deviations for a number of determinands. For each determinand, only one fixed value is given, but the recommendations are concerned mainly with narrow concentration ranges around values specified in water-quality standards.

The use of the above target values for random (precision) and systematic (bias) error is described in the following sections.

2.3.6 *Provisos to the approach in sections* 2.3.1–2.3.5

In the above approach, a few points should be noted.

(*i*) There is a slight numerical inconsistency between the values of L and the maximum tolerable standard deviation at low concentrations. Thus, if results are normally distributed with a standard deviation, σ_B, independent of concentration at low concentrations, the limit of detection is given by $3.29\sigma_B$ (probabilities of errors of the first and second kinds[14,15] are both 0.05). Hence, at low concentrations the maximum tolerable standard deviation (specified as $0.25L$) is $0.82\sigma_B$, *i.e.*, it is smaller than the standard deviation achieved, σ_B. This inconsistency could be eliminated, for example, by selecting slightly different probability levels to define the limit of detection. However, such complication is considered unnecessary because of the uncertainties involved in estimating σ_B and the fact that the targets for errors should not usually be regarded as precisely fixed constants. If the target values are interpreted with common sense, no difficulty is caused by the above inconsistency.

(*ii*) The limit of detection may not be directly relevant to certain determinands, *e.g.*, pH value. When this is so, the expressions for tolerable errors will usually need to specify only fixed percentage or absolute errors.

(*iii*) As mentioned above, the numerical values of p and L should be chosen appropriately for each scheme. However, as those values are reduced, the analytical effort and cost needed to achieve them will generally increase and, at some point, will become impracticably large (see also section 2.9.3). Care is required, therefore, in specifying errors that are both tolerable and achievable. The target values in the Harmonised Monitoring Scheme may seem rather large, but experience of the approach in this paper suggests that they are both useful and realistic. It is interesting that McFarren *et al.*,[16] in considering the results from many inter-laboratory studies of different analytical methods, concluded that for only a relatively small fraction of methods studied were total errors* of 25% or less achieved.

(*iv*) In specifying a particular method in order to define a non-specific determinand, care is also necessary to ensure that the method selected is capable of the required accuracy and limit of detection.

2.4 Choice of Analytical Methods

This is the most important stage of all because an inappropriate choice will not only prevent achievement of the required accuracy but is also likely to cause much wasted time and effort. Accordingly, the choice of methods is considered in some detail below.

It sometimes appears to be thought that comparable results can simply be achieved by ensuring that all laboratories use the same method for a particular determinand. However, innumerable experimental studies have shown that this approach does not necessarily control the errors of all laboratories; see, for example, the compilation of inter-laboratory tests given by McFarren *et al.*[16] In addition, use of the same method by all laboratories penalises

* The definition of "total error" used by McFarren *et al.* is similar to but not identical with that in this paper; see also reference 17.

those able to take advantage of analytical advances, *e.g.*, by using new methods or instruments. This approach is not, therefore, generally recommended except when required by the definition of a determinand (see section 2.3.2).

In principle, the approach recommended here is simple, in that each laboratory merely has to select a method capable of the required accuracy. Of course, other factors such as the speed and simplicity of a method are frequently important and should be consider d carefully when choosing a method from those capable of the required accuracy. In applying this approach, several aspects need careful consideration and those of general importance are summarised below.

2.4.1 *Definition of determinands by specifying a particular analytical method*

The methods to be used for certain determinands may already have been defined (see preceding section). When this is so, there is, in principle, no choice to be made, and it is necessary only to ensure that all laboratories follow essentially the same procedure (see section 2.5). This approach has been used in the Harmonised Monitoring Scheme for suspended solids and biochemical oxygen demand. It is also possible to allow the use of alternative methods for a particular determinand if they are first shown to give results in satisfactory agreement with the specified method. This latter approach is basically sound, but can lead to problems because of the experimental difficulties involved in proving satisfactory agreement, particularly when the samples of interest are of markedly differing compositions.

2.4.2 *Determinands that are defined chemical species*

For any determinand other than those in section 2.4.1, the method should be selected from among those known to be capable of the required performance and for which a thorough investigation of the effects of relevant experimental parameters has been made.[5,18,19] If only one such method exists, it is technically desirable for all laboratories to use that method, but see section 2.4.4. When several suitable methods are available for a particular determinand, each laboratory is free to choose the one that it prefers. In making that choice it is useful to select methods capable of rather smaller errors than the maximum tolerable errors defined above. This will tend to ensure a safety margin and to reduce the need for frequent corrective action to reduce intolerably large errors. Particular attention should be paid to sources of bias when selecting a method[5,11,18,20] because in many situations the choice of a suitable method is the main control on bias in the analytical results. Notwithstanding the specified tolerable bias, it is recommended that whenever possible the aim should be to select methods with negligible sources of bias.

2.4.3 *Comparing the required and published performances of analytical methods*

In comparing the published performances of analytical methods with the required limit of detection and maximum tolerable standard deviation and bias, care is necessary because the definitions of these characteristics and the methods used to estimate and report them often differ from one publication to another.[18–22] This potential problem is readily eliminated if authors make clear the definitions and procedures relevant to their quoted performance characteristics and if they give unambiguous information on the magnitudes of errors. Within the UK one particular approach to the definition and tabulation of performance characteristics has been developed by the Central Electricity Research Laboratories.[5,19–22] This approach has been followed by the Water Research Centre and recently by a committee* concerned with the selection, evaluation and publication of methods for the analysis of a wide range of waters, effluents and other materials.[23] An example of such a tabulation is given in reference 24. Essentially the same approach is followed in the Harmonised Monitoring Scheme.

In comparing published and required values of standard deviations and limits of detection, careful consideration of the sensitivity and discrimination of the analytical-measurement system is also essential. For example, a published method may have used an instrument

* The Standing Committee of Analysts to Review Standard Methods for Quality Control of the Water Cycle, established by the Department of the Environment and the National Water Council.

of better discrimination (ability to make finer readings of the instrument scale) than that available in another laboratory. If instrumental errors govern precision, the latter laboratory will be unable to achieve the published precision. Thus, in selecting methods each laboratory should ensure that the sensitivity and discrimination of its preferred method will allow the required precision to be achieved.

2.4.4 *Availability of suitable analytical methods*

The approach in section 2.4.2 cannot always be followed in the Harmonised Monitoring Scheme for two reasons. Firstly, thoroughly evaluated and characterised methods are not at present available for all determinands. In the absence of such methods, there is much to be said for delaying attempts to achieve comparable results, but the need for information on water quality commonly requires some attempt to control analytical errors. Fortunately, this problem is gradually being eliminated by the work of the many laboratories and organisations concerned with the development of analytical methods. Secondly, the staff and/or equipment of a laboratory may necessitate the use of a method whose capability of achieving the required accuracy is at least partially in doubt. Therefore, the use of methods not known to be suitable requires consideration.

If a laboratory has to select an analytical method that has not been thoroughly evaluated, critical assessment of its likely errors is desirable.[5,18,20] Certain sources of bias can sometimes be readily eliminated, *e.g.*, in the blank and calibration procedures.[5,18,20] Other likely sources of bias and the method's ability to achieve the desired precision should then be assessed. To aid uniformity among laboratories in such appraisals, it is useful for the co-ordinating laboratory to make similar assessments of all such methods; this approach is followed for all determinands in the Harmonised Monitoring Scheme. Whenever possible, each laboratory concerned should then make any preliminary tests needed to estimate the unquantified errors.[1,5] This seldom presents great difficulty if only the precision is in doubt. However, if, as is common, little is known of interference effects,[11] the task is much more difficult because of the large amount of work needed for a reasonably thorough investigation of such errors,[20] particularly for samples whose composition is as variable as river waters. Other sources of bias[20] may also not be simple to determine. Such uncertainties and the effort needed to resolve them are a powerful argument for the approach in section 2.4.2. In the present context, however, experience shows that routine laboratories cannot usually make all the required tests. Hence, all that can be done in the short term is for a laboratory to make as many tests as possible, and then to proceed to subsequent stages of Fig. 1; the latter stages may detect certain sources of bias, *e.g.*, in the tests described in section 2.9. In the longer term, the aim should be either to complete any tests on errors or to replace the method with one whose performance is better established.

2.4.5 *Importance of sample preservation procedures*

As mentioned above, stability of the concentration of a determinand between sampling and analysis must be ensured, but the procedures used to that end can affect the performance of a subsequent analytical method. It is desirable, therefore, to select only methods that include, as an integral part of the total procedure, all aspects relevant to sample preservation, *e.g.*, details of sample containers and preserving reagents.

2.5 Written Descriptions of Analytical Methods

Virtually all publications concerned with analytical quality control stress the importance of detailed written descriptions of analytical methods. In addition to the clear need for such descriptions in each participating laboratory, they are also essential in assessing likely sources of error (see section 2.4.4).

There are many possible formats that such descriptions can take and an international standard is available.[25] It suffices here to suggest that the aim should be to specify the entire experimental procedure in such detail that, if it were faithfully followed, an inexperienced analyst would be able reliably to achieve adequate accuracy. Relevant points have been reviewed by many workers; see, for example, references 18 and 19. The type of format favoured by the author is illustrated in references 5 and 24.

It is also essential to do everything possible to ensure that the method is followed exactly in all subsequent work, at least until tests have demonstrated the validity of any contemplated changes. In considering such changes, great caution is generally desirable; even apparently minor procedural details can sometimes have unsuspectedly large effects, particularly in trace analysis. One of the aims of subsequent stages of Fig. 1 is to detect any deterioration in the accuracy of routine analytical results caused by unsuspected changes in procedure.

2.6 Ensuring Adequate Within-laboratory Precision

Three main considerations lead to this and the three subsequent stages of Fig. 1.

(*i*) Many factors, other than the written description of a method, can affect the precision of analytical results. Such factors include the purities of reagents, the reliabilities of instruments, the abilities of analysts and the degree of contamination problems. Therefore, the fact that one or more laboratories have achieved adequate precision when using a given method does not guarantee this for another laboratory. Experimental estimates of precision should, therefore, be obtained by each laboratory.

(*ii*) Direct tests of between-laboratory bias are usually not easy to arrange on a frequent basis. There is an advantage, therefore, in attempting to control as many sources of error as possible by tests that can be made independently by each laboratory.

(*iii*) The number of replicate tests needed to detect bias of a given magnitude is governed by the standard deviation of results.[16,26] It is useful, therefore, to ensure that all laboratories achieve adequately small standard deviations before making tests of bias.

On this basis, estimation of within-laboratory precision by all laboratories is the first of the experimental stages in Fig. 1.

2.6.1 *Important factors to be considered in designing tests of precision*

In deciding an appropriate experimental design for such tests, several desiderata are generally relevant.[1,5,21]

(*i*) Precision commonly depends on the concentration of the determinand. Therefore, except when interest is attached only to a narrow concentration range,[9] estimates of standard deviation should be obtained for at least two concentrations.[21] Further, whenever the limit of detection is of interest, the within-batch standard deviation of blank determinations should usually be estimated.[14,15,22]

(*ii*) Precision commonly worsens as the time period over which the tests are made is increased. As it is the precision of routine analytical results that is of interest, the tests should be spread over a number of occasions rather than being made on only one occasion.

(*iii*) Precision may depend on the nature of the sample analysed, and in particular real samples (*e.g.*, river waters) may give worse precision than standard solutions. However, standard solutions are of general value in analytical quality control[1,5,11] and tests of precision for both standards and real samples are, therefore, useful (see section 2.8).

(*iv*) Precision is assessed from replicate results for a given sample; these results should be independent of each other.[21] The determinand concentration in the sample must also be the same on each occasion it is analysed.

(*v*) As the number of tests and samples is increased, more information is obtained and the uncertainties of the estimated standard deviations tend to decrease. This has generally to be balanced against the amount of effort required and available for the tests.

Many experimental designs for estimating precision have been described and any may be used, provided valid estimates of the required information are obtained. The design normally followed in the Harmonised Monitoring Scheme aims to meet the above desiderata,[5,21] and is summarised in section 2.6.2; experience to date suggests the design to be of general value.

2.6.2 *Typical experimental design*

In each of m batches of analyses (no more than one batch in any one day), n portions of each of the following are analysed in random order: (*i*) blank determination; (*ii*) standard solution of concentration 0.1 C_u (C_u = upper concentration limit of the analytical method); (*iii*)

standard solution of concentration 0.9 C_u; (*iv*) a sample of river water; and (*v*) the same as (*iv*) but with an accurately known addition (equivalent to 0.5 C_u) of the determinand.

All analyses are made exactly as for normal samples by the same experienced analyst. The values of m and n are usually 10 and 2, respectively, but other values can be used.[5,21] Special consideration of the value for n may be needed if the analytical method specifies analysis of more than one portion of a sample and/or blank in order to produce an analytical result for a sample. The value $n = 2$, mentioned above, applies to the usual situation where only one portion of a sample and a blank is specified in the method. Other details are given elsewhere.[5,21] Precautions are necessary when the determinand is unstable, and these are summarised in the Appendix.

2.6.3 *Calculations and comparison of required and achieved performances*

At the end of the tests, for each of the solutions (*ii*)–(*v*) the 20 analytical results are analysed statistically[5,21] to obtain estimates of the within-batch (s_w), between-batch (s_b) and total (s_t) standard deviations, where $s_t^2 = s_w^2 + s_b^2$. The results for the blanks are used to provide an estimate of its within-batch standard deviation (in concentration units). For each of the solutions (*ii*)–(*v*), the value of s_t is compared (variance-ratio test) with the maximum tolerable standard deviation at the corresponding concentration of the determinand. The precision is then accepted as adequate if s_t is not significantly greater (0.05 probability level) than the target value (see Appendix). Similarly, the limit of detection can be calculated from the value of s_w for the blank and then compared with the target value (see Appendix). If all targets are achieved, it can be concluded (but see the Appendix) that the precision is adequate. If one or more targets are not achieved, sources of imprecision should be sought, reduced and the tests then repeated. When this is necessary, the values of s_w and s_b for a particular solution may help to indicate the likely source of error,[5,21] and this is one of the main reasons for choosing $n = 2$, *i.e.*, this allows separate estimations of s_w, s_b and s_t. When $n = 1$, only s_t can be estimated.

In addition to the above estimates of precision, the recovery of the determinand added to solution (*v*) is also calculated. This recovery will generally differ from 100%, even in the absence of bias, as a result of random errors, and a criterion is, therefore, needed to decide whether or not the observed recovery is to be considered as adequate. In the Harmonised Monitoring Scheme, this criterion is that the recovery should not differ significantly (*t*-test, 0.05 probability level) from the range 95–105%. Again, if the results are unsatisfactory, the cause should be sought, reduced and the tests then repeated.

Finally, the results from solutions (*i*)–(*iii*) allow the analytical sensitivity* to be compared with that expected for the method. Other aspects such as the linearity of the calibration graph should also be checked. If such parameters depart appreciably from those expected for the method, it is as well to seek and, if necessary, eliminate the reasons for such differences as they may indicate mis-application of the method or other problems.

When all the above aspects can be regarded as satisfactory, the next stage in Fig. 1 (section 2.7) is started.

2.6.4 *Co-ordination of tests*

One general point on these and subsequent tests in laboratories is worth noting here. To ensure that all laboratories carry out the agreed tests and calculations uniformly, the co-ordinating laboratory should prepare, (*i*) detailed descriptions of all aspects of the tests for each determinand, and (*ii*) suitably designed forms on which laboratories can enter the results from the tests. These forms can also, and in the Harmonised Monitoring Scheme do, include detailed instructions on how to make the various statistical calculations involved. Experience shows that if such details and forms are not supplied to all laboratories, some participants may make inappropriate tests and calculations and this may invalidate their work. The co-ordinating laboratory is also likely to meet problems in attempting to check and collate all results if they have not been calculated and presented in a uniform format.

* The term sensitivity is used here to denote the rate of change of the analytical response with determinand concentration. Thus, when the response is directly proportional to concentration, the sensitivity is the slope of the calibration graph.

2.7 Accuracy of Standard Solutions

This stage is intended to ensure that the standard solutions (used for calibration purposes) of all laboratories are in satisfactory agreement and hence do not cause important between-laboratory bias. In principle, this aim could be achieved by the co-ordinating laboratory providing the necessary standard solutions for all laboratories. In practice, however, this approach is not favoured because it is cumbersome, liable to practical difficulties and very demanding of effort from the co-ordinating laboratory. A better approach, and one which is used in the Harmonised Monitoring Scheme, is as follows.

All laboratories prepare their standards as prescribed in their analytical methods, and the co-ordinating laboratory distributes a portion of its own concentrated standard solution for the determinand under study to each laboratory. Of course, considerable care is essential to ensure that the distributed standard has a negligible error. In water analysis, particular care is often required to ensure that the concentration of the determinand in the distributed solution is stable for an adequate time period and that the containers used for the standard do not cause contamination. Such problems are usually minimised by distributing relatively concentrated standard solutions; this also aids transport arrangements because relatively small volumes of such solutions suffice for the subsequent tests. Each laboratory then compares the concentrations of its own standard with the distributed standard in the following way.

Both standard solutions are diluted accurately to the same concentration, this concentration being that for which the tests in section 2.6.2 have indicated the smallest within-batch relative standard deviation*; this concentration is usually at or near C_u. Then q portions of each diluted standard solution and one blank determination are analysed in one batch of analysis in a prescribed order† and the mean results for each standard compared using a t-test. The value of q is calculated by each laboratory to allow the experiment to have the desired statistical power of detecting a difference of d% between the two standards.[26] In the Harmonised Monitoring Scheme, $d = 2$, and the probabilities of errors of the first and second kinds are both set at 0.05.

If any laboratory finds that its standard solution is in error by more than d%, the cause is sought and reduced and the tests are repeated. When a laboratory's standard is in satisfactory agreement with the distributed standard, the next stage in Fig. 1 (section 2.8) is started.

2.8 Analytical Quality-Control Charts

Even though a laboratory has satisfactorily completed the two preceding stages, this by no means guarantees that its accuracy will remain permanently satisfactory. It is essential, therefore, for each laboratory to maintain continuing checks that its errors remain adequately small. A widely recommended and used approach[1,5,6,8,9,12] is to make special control tests in each batch of the normal analyses and to plot the results immediately they are obtained on statistical quality-control charts. If the results from the control tests indicate that the accuracy has worsened appreciably, further analysis of samples is stopped until the source of the increased error has been found and eliminated. This use of quality-control charts is recommended here and is followed in the Harmonised Monitoring Scheme. Several important aspects in using such charts for analytical quality control are summarised in sections 2.8.1–2.8.6. It will be seen that various uncertainties arise, and this emphasises the desirability of laboratories seeking to obtain accuracy rather better than that expressed by the maximum tolerable errors.

2.8.1 *Basis of quality-control charts*

For those who may be unfamiliar with the concepts underlying statistical quality-control charts, most general statistical texts deal with the topic; see, for example, references 27 and 28. The use of these charts in analysis has also been described by many workers.[5,6,8,12,29]

* The relative standard deviation signifies here the standard deviation for a particular concentration expressed as a percentage of that concentration.

† A random order is usually suitable but systematic orders may be preferable if the analytical response can drift continually in one direction.

2.8.2 *Choice of type of control test*

In general, no single control test can check all possible sources of error. For example, if a standard solution is analysed in each batch of analyses, this gives information on precision and certain sources of bias, but clearly gives no direct information on the accuracy of sample analysis. Information on the precision for samples can be obtained by analysing two or more portions of a sample in each batch, but this gives no information on bias. Recovery tests using samples may be useful in providing information on certain, though not all, sources of bias, but are likely to present problems in assessing precision. Ideally, all these and any other relevant control tests would be made routinely, but a compromise with the effort required is usually necessary. Each situation should, therefore, be assessed individually to decide the most appropriate control test(s) for a given determinand. This has been discussed in more detail elsewhere,[30] and the results from the preceding tests of within-laboratory precision are of value in deciding which control tests to use. For most determinands, the single best control test is usually considered to be the analysis of a standard solution, particularly when the initial tests of precision have shown essentially the same precision for standard solutions and samples. Of course when this control is used, it is essential to ensure that the concentration of the standard solution has negligible error on each occasion it is analysed.

Another factor frequently relevant to the choice of the control test(s) is the extent to which precision depends on the concentration of the determinand. If, for example, the standard deviation of results increases markedly with concentration, there is some problem in deciding the best single concentration to use for a control standard. Of course, if only a narrow range of concentrations exists or is of interest, the mid-point of that range will usually be a suitable value for a control standard. Otherwise, it is preferable to use two control standards, one near the lower limit of the concentration range of the method, and the second near the upper limit. If the effort necessary for both standards is not available, the use of the upper concentration is, on balance, favoured. It should be noted that problems also arise in the construction and interpretation of control charts for the two other types of control test mentioned in the preceding paragraph, whenever precision depends on concentration and the concentrations in samples cover a wide range.[30] Various devices can be used to reduce such problems. For example, one control chart for each of a number of relatively narrow concentration ranges can be used for each type of control test, the results being plotted on the appropriate chart.

2.8.3 *Frequency distribution followed by results*

To allow exact interpretation of the results of control tests, the nature of the probability distribution followed by the results must be known; it is convenient and common to assume the normal distribution. To ensure that the results of control tests closely follow this distribution, statistical texts usually recommend that, for example, the control test is replicated in each batch and the mean of the results is plotted as the control point for each batch. This approach is desirable, but again the effort available may only allow single control tests in a batch. When this is so, it is still of value to use control charts, but it must be accepted that the control charts cannot be exactly interpreted without further information on the nature of the probability distribution of results. In practice, this is not considered an important objection.

2.8.4 *Types of quality-control chart*

In the Harmonised Monitoring Scheme, the results from control tests are plotted on Shewhart-type control charts.[27–29] In recent years, another type of chart, the CUSUM quality-control chart, has come into common use and is said to be advantageous in allowing more rapid detection of changes of accuracy.[27,31] This latter chart has been recommended for use in clinical chemistry[1,32] and has been included in one manual on analytical quality control in water analysis.[6] An investigation of the use of CUSUM charts in water analysis is to be made in the author's laboratory. At present, however, it is considered that there is little to choose between the two types of chart for analytical quality control, given the various uncertainties involved in the practical use of control charts.

2.8.5 *Construction of Shewhart-type quality-control charts*

Before a control chart can be constructed, an estimate of the standard deviation of the results of control tests is required to allow insertion of the warning and action limits. Ideally, this estimate would have a large number of degrees of freedom,[27–29] but insistence on this would delay construction of the chart for a relatively long period while the necessary tests were being made. In the Harmonised Monitoring Scheme, therefore, a preliminary control chart is constructed as soon as a laboratory's standard solution has been shown to be adequately accurate. The estimates of standard deviation obtained from the tests of precision are used for this purpose. Such estimates have at least 9, and usually more, degrees of freedom (see the Appendix), but revised estimates should be obtained (and the warning and action limits on the chart adjusted accordingly) as the results from control tests accumulate.[30] Particular care is needed in using the chart initially, but it is of value for laboratories to have a sequential record of the control tests from the outset, especially as an appreciable time period may elapse before the next stage (section 2.9) of Fig. 1.

2.8.6 *Anonymity of control solutions*

There is value, but commonly difficulty, in arranging that the analysts concerned are unaware of which solutions are controls.[9] In the Harmonised Monitoring Scheme, individual laboratories decide whether or not to adopt this approach. The importance of this aspect is much reduced if all analytical staff are brought into a quality-control scheme properly, with full understanding of its purposes.

2.9 Checking Between-laboratory Bias

As soon as all laboratories have satisfactorily completed the previous stages, this final stage is begun. The approach adopted is very simple. Portions of one or more standard solutions and/or samples are distributed to each laboratory, which then makes sufficient replicate determinations on each solution to allow detection of a bias equal to the maximum tolerable value. The bias is assessed separately for each solution because both bias and precision may depend on the concentration of the determinand and on the nature and general composition of the solution. Other approaches to between-laboratory tests have been described, which may allow some economy of effort or a greater amount of information for the same effort; such approaches have been briefly reviewed.[7] However, these advantages are gained by making assumptions, such as equal precision in all laboratories. For present purposes, it is considered essential not to make such assumptions, and the approach described below is followed.

The co-ordinating laboratory prepares and distributes appropriate solutions to all laboratories; only a broad indication of the concentrations of these solutions is given to the laboratories. Each laboratory then analyses w portions of each solution, the normal control test(s) (see section 2.8) being included in each batch of analysis. The results obtained are reported to the co-ordinating laboratory for assessment of the bias for each laboratory and solution. Any laboratories with an unacceptably large bias are informed so that they can seek and reduce the cause before undertaking further tests of bias. Further details relevant to this approach are given below.

2.9.1 *Solutions to be used*

The main sources of bias needing to be checked are those arising from the analysis of samples rather than standard solutions. Emphasis should whenever possible, therefore, be given to the distribution of the former. Nevertheless, it is useful to include at least one standard solution so that the true concentration of at least one distributed solution is known. The distributed solutions should also cover the range of determinand concentrations of interest, and in general, the more types of sample that can be included the better. In the Harmonised Monitoring Scheme, the general aim is to distribute one standard solution near the middle of the concentration range of interest, and two samples of river water of different types with concentrations near the lower and upper concentrations of interest. Whenever possible (see section 2.9.2), these solutions are distributed at the above concentrations so that their dilution before analysis is not necessary; such dilution may prevent detection of certain sources of bias.

2.9.2 *Stability of distributed solutions*

It is essential that the co-ordinating laboratory takes great care to ensure that each laboratory receives essentially identical portions of each of the distributed solutions. Of particular importance in water analysis are the cleanliness of solution containers and the stability of the determinand. Preliminary tests in the co-ordinating laboratory are often desirable to ensure this identity of the distributed solutions. In water analysis, many determinands are so unstable that distribution of samples without special treatment is impossible. When this is so, the use of preserving reagents can be of value, but the co-ordinating laboratory should first ensure that a reagent it proposes to use is compatible with the analytical methods used by all laboratories. One approach commonly recommended to overcome such problems is to distribute a stable, standard solution with a sample, the former being used to spike a portion of the latter before analysis. The difference between the results for the spiked and unspiked portions is then used to assess the bias. This procedure can be of value but should be used with caution because it may not detect those sources of bias whose magnitudes are independent of the concentration of the determinand (see section 2.9.4). Therefore, the best approach needs to be decided individually for each study; the procedures used in the Harmonised Monitoring Scheme for particular determinands will be described in subsequent papers.

2.9.3 *Number of analyses required on each solution*

The number of replicate analyses, w, required on each solution depends on the ratio B/σ, where B is the maximum tolerable bias and σ is the standard deviation of results.

Davies[26] describes the calculation of w for different values of B/σ, for various probability levels and when σ is not known exactly. In the Harmonised Monitoring Scheme, an approximate value for w to be used by all laboratories is obtained by setting B and σ_t equal to their maximum tolerable values. The ratio B/σ_t is, therefore, 2.0, and the corresponding value of w (0.05 probability of errors of the first and second kind) is approximately 3. However, it is necessary to increase this value somewhat because only an estimate of σ_t is available.[26] At the same time, there will be a tendency for σ_t to be less than the maximum tolerable value. Hence, for convenience, the approximation $w = 5$ is used for each solution; experience shows this to be a reasonable approach. The precision achieved by certain laboratories may be substantially better than the maximum tolerable value so that a value for w smaller than 5 could be used. However, the use of $w = 5$ throughout is preferred because it provides better estimates of precision from these tests (see section 2.9.5) and also ensures better ability to detect bias by those laboratories with better precision.

It is also worth noting that the values of the maximum tolerable bias and standard deviation have a marked effect on the number of tests needed to detect such bias. For example, suppose that B is 5%, σ_t is 5% and that a laboratory just achieves the required precision. The number of tests required on each solution would, on the above basis, be approximately 15, a number that, for several reasons, would often be impracticable.

Whenever possible, one portion of each of the distributed solutions is analysed on each of five days. This approach makes some allowance for the possibility that bias can vary from day to day, and thus provides an estimate of the average bias; see also section 2.9.5. However, if the stability of a distributed solution or the convenience of the laboratories require, all replicate analyses may be made on one day without great effect on the value of the information.

2.9.4 *Calculation of bias for each laboratory*

In the Harmonised Monitoring Scheme, for each solution and laboratory, the mean and its 90% confidence limits, $\bar{x} \pm h$, are calculated from the five individual results. The upper limit for the true bias (95% confidence level) is then calculated by the co-ordinating laboratory as $\bar{x} + h - T$ if $\bar{x} > T$ (where T is the true value for the distributed solution) or as $\bar{x} - h - T$ if $\bar{x} < T$. These expressions apply when the value of T is such that the maximum tolerable bias is a fixed concentration (see section 2.3). For greater values of T where the target is a fixed percentage of T, the corresponding expressions for bias become $100(\bar{x} + h - T)/T$ and $100(\bar{x} - h - T)/T\%$. If the estimated bias is greater than the maximum tolerable value, laboratories are informed as soon as possible so that, in general,

possible causes of the bias can be sought and eliminated. In practice, this assessment of bias also includes some subjective consideration. For example, a laboratory whose bias marginally exceeded the maximum tolerable value for one solution but was well within the target for other solutions is usually considered acceptable. On the other hand, a laboratory only just within the target for all solutions may well have need to check possible sources of bias.

In this approach, T is equated to the known concentration of the distributed standard solution. For samples of river water, the value of T is not generally known and an approximate value must be used. In the Harmonised Monitoring Scheme, this value has generally been equated to the mean of results from all laboratories. This approach is considered reasonable given the preceding stages of Fig. 1 and the number of laboratories (11) involved,* but it is, of course, not necessarily correct. Careful consideration of the results from each test and of possible sources of bias in the analytical methods used is generally essential before assigning a value to T.[7] This problem can sometimes be much reduced if a sample can be obtained with a negligible concentration of the determinand but typical in all other respects. An accurately known amount of the determinand can then be added by the co-ordinating laboratory before distribution of portions of the spiked solution. In this situation, the value of T can be determined with reasonably small error and this approach has been useful in connection with the determination of lead in drinking water.[34] Similarly, T would be known if bias is assessed from the difference between the results for spiked and unspiked portions of a distributed solution (see section 2.9.2).

2.9.5 *Estimates of precision*

The results from these tests also provide estimates of the standard deviation, s_t, of analytical results for each of the distributed solutions. These estimates are poor in that they have only four degrees of freedom, but it is useful to compare these estimates with the target values as in the tests of within-laboratory precision. If there has been any deterioration of precision not revealed by the control chart(s), the upper limit for bias may be largely governed by the imprecision ($\pm h$) of the mean result rather than by bias.

2.9.6 *Need for further inter-laboratory tests of bias*

On satisfactory completion of this final stage of Fig. 1, it is provisionally taken that laboratories have achieved the required accuracy. However, precision and bias at this stage have been estimated for few sample types and both parameters may also deteriorate subsequently. As explained above, the use of control tests will help to ensure that precision and certain sources of bias remain satisfactory, but it is also considered essential to repeat the tests of between-laboratory bias regularly. As always, the more frequent such tests and the greater the number of samples included in them, the better will be the control. The effort involved is, however, substantial, and at present in the Harmonised Monitoring Scheme, the tests are repeated at approximately 6-monthly intervals for each of the determinands studied to date. Further, only one sample is distributed in each repeat test, but the aim is to use samples of types different to those distributed in previous tests.

3 Conclusions

The discussion above has shown that close attention must be paid to many different aspects of analysis if the accuracy of routine analytical results is to meet a specified and reasonable value. It is worth stressing that virtually all the many points and suggestions mentioned in this long paper have been included because they have caused problems in one or more groups of laboratories with which the author has been involved.

To overcome such problems permanently, a systematic approach is essential, and the scheme described here (summarised in Fig. 1) represents one such approach that has been found successful in the analysis of river and other waters. It is considered that the principles of this approach are of general value and validity, but, of course, many detailed modifications are possible. The fact that the scheme is involved and unlikely to achieve rapid progress is

* The accuracy of the value used for T will, all other things being equal, improve as the number of laboratories increases.

a disadvantage. However, it seems to the author that it is only by a scheme of this nature that any progress will be achieved on a permanent and sound basis. Clearly, there is no substitute for detailed consideration of each particular application and this may well suggest simplifications of value. Nevertheless, such simplifications should only be undertaken after very careful consideration; experience shows that lack of success and wasted effort can result if short cuts are taken on insufficient evidence.

Clearly, effort is required to implement analytical quality control, and the amount depends on the required accuracy, the analytical methods and the degree of control considered appropriate. As a broad indication, suggestions have been made that 10–20%[11] or 15–20%[6] of an analyst's time should be devoted to quality-control work. This proportion may seem rather large, and may be difficult to achieve in initially establishing an analytical quality-control scheme in a laboratory. Nevertheless, such effort is considered a reasonable goal though initiation of control schemes should not be prevented if only a smaller amount of effort is available initially. In general, a little control is better than none.

Finally, it is worth making the obvious point that it is not generally possible to obtain direct estimates of precision and bias for every sample that laboratories analyse. Hence, though in the author's opinion analytical quality control should be an integral part of the work of a laboratory, it cannot prove the accuracy of every result. Rather, it provides valuable confirmatory evidence of the adequacy of all the various means adopted by the analyst to ensure the required accuracy. In other words, the continuing and critical assessment by the analyst of all that he does remains, as always, of prime importance.

Many colleagues have contributed to many aspects of the approach described in this paper. The author is grateful to them all, but particular thanks are due to Messrs. R. V. Cheeseman, D. J. Dewey, I. R. Morrison and W. J. Wyse and, for much guidance and advice on statistical aspects, to Messrs. W. J. Allum, J. C. Ellis, R. E. Fry and R. F. Lacey. Thanks are also due to the author's many colleagues in the Department of the Environment, Water Authorities and River Purification Boards who operate the Harmonised Monitoring Scheme. Finally, the author is also grateful to the Director of the Water Research Centre for permission to publish this paper.

Appendix

A few points of detail concerning the tests of within-laboratory precision are mentioned below.

(*a*) The degrees of freedom of the estimates s_w and s_b are $m(n-1)$ and $(m-1)$, respectively. The concept of degrees of freedom is not strictly applicable to the combined estimate, s_t, but a reasonable approximation is to assign f degrees of freedom to s_t, where f is the integer nearest to the value of

$$\frac{m\,(m-1)\,[M_1 + (n-1)\,M_0]^2}{m\,M_1^2 + (m-1)\,(n-1)\,M_0^2}$$

using the notation in reference 21.

(*b*) In comparing an experimental estimate of a standard deviation with a target value by the variance-ratio test, the target standard deviation has an infinite number of degrees of freedom.

(*c*) For a given solution, the precision of a laboratory is considered acceptable if s_t is not significantly greater (0.05 probability level) than the target value. Such values of s_t can be obtained when the population standard deviation, σ_t, is greater than the target value, *i.e.*, the probability of an error of the second kind is much greater than 0.05. If desired this probability can be decreased by increasing the number of replicate analyses used to estimate s_t[26,27] but the number corresponding to a probability of 0.05 will normally be impracticable. Three points reduce, though they do not eliminate, this problem.

(*i*) An estimate, s_t, is obtained for each of several solutions, and this provides an opportunity to judge whether or not there is a general tendency for all s_t values to approach or exceed the targets.

(*ii*) Subsequent tests (see section 2.8) will provide more precise estimates of σ_t.

(*iii*) By aiming to use methods capable of rather better precision than required, there will be a tendency to obtain σ_t values less than the targets.

(*d*) To ensure valid estimates of σ_b and σ_t, the concentration of the determinand in a given solution must be the same for all batches of analyses. When determinands are unstable, this condition can be satisfied for standard solutions by using freshly prepared solutions for each batch. However, for samples no direct estimates of σ_b and σ_t are possible and the following approximate approach is followed. A freshly collected sample is used for each batch, these *m* samples being selected so that the determinand concentration is approximately the same in each. Alternatively, the same sample can be used for all batches provided its concentration of determinand changes by no more than approximately 20%. On completion of the *m* batches, s_w, s_b and s_t are calculated for standard solutions and s_w is calculated for samples. The assumption is then made that the between-batch random error is due only to uncorrected variations in the calibration graph from batch to batch. On this basis and when the analytical response is directly proportional to the concentration of the determinand, σ_b is independent of the type of solution and is also directly proportional to concentration, *i.e.*, $\sigma_b = kC$. An approximate value of *k* can, therefore be obtained from values of s_b for standard solutions. Thus, an approximate value for s_b for a sample can be obtained from its mean concentration and the estimated value of *k*. Finally, s_t for a sample can then be calculated from $s_t = \sqrt{(s_w^2 + s_b^2)}$.

(*e*) The limit of detection (0.05 probability of errors of the first and second kinds) is given by $4.65\sigma_w$, where σ_w is the within-batch standard deviation of blank determinations (in concentration units),[14,15] provided that:

(*i*) an analytical result is obtained by making one measurement each of a blank and a sample, the apparent concentration of the blank being subtracted (in fact or in effect) from that of the sample;

(*ii*) the apparent concentrations of any solution of low concentration follow the normal distribution; and

(*iii*) the within-batch standard deviation of the apparent concentrations for any solution of low concentration is independent of the concentration of the determinand and is the same for blanks and samples.

If any one of these conditions is not satisfied, other expressions for the limit of detection are required.[15]

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Received *September 11th*, 1978
Accepted *November 2nd*, 1978

Analyst, April, 1979, Vol. 104, pp. 290–298

Accuracy of Determination of Chloride in River Waters: Analytical Quality Control in the Harmonised Monitoring Scheme

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The Department of the Environment, in collaboration with the Regional Water Authorities, has initiated a Scheme for the Harmonised Monitoring of the Quality of Inland Fresh Waters in England and Wales. The Scottish Development Department has introduced a similar scheme in Scotland in collaboration with the Scottish River Purification Boards. To achieve the required comparability of results from all laboratories involved, each laboratory takes part in an analytical quality control (AQC) scheme; this work is co-ordinated by the Water Research Centre. The general approach adopted to AQC has been described, and this paper presents the tests made and results obtained in the determination of chloride in river waters. Broadly, each of the ten participating laboratories achieved total errors not greater than ±20% of the chloride concentration for concentrations greater than 5 mg l^{-1} of chloride.

Keywords: *River-water analysis; chloride determination; accuracy of results; inter-laboratory comparability; analytical quality control*

The scheme for the Harmonised Monitoring of the Quality of Inland Fresh Water has been described recently in detail.[1] It is intended to provide objective data on river water quality so that accurate assessments can be made of long-term trends in the qualities of rivers and of the amount of material discharged by them to the sea. The Scheme complements monitoring carried out for regional or local purposes and one of its essential aims is to achieve comparability of the results from all participating laboratories. To that end, special investigations have been made to establish suitable locations for sampling and to define the necessary sampling frequencies. Sampling procedures have been harmonised, and to ensure that subsequent analyses do not introduce unacceptably large errors, each participating laboratory carries out a specially designed programme of tests to ensure that their analytical results are of adequate accuracy for the Scheme. The Water Research Centre (WRC) is under contract to the Department of the Environment to advise on and co-ordinate this analytical quality-control (AQC) programme.

The need for, and details of an approach to, a planned AQC system for this and similar schemes have been discussed in some detail elsewhere in this issue.[2] In view of the growing interest in achieving comparable results from each of a number of laboratories, it was thought useful to describe the AQC work for the Harmonised Monitoring Scheme and to present the results obtained for different determinands. This paper considers the determination of chloride and subsequent papers will deal with other determinands of general importance in rivers. Chloride was chosen for the first study of the Committee because serious problems in achieving the required accuracy were not expected and it was considered, therefore, that this would facilitate the introduction of the AQC programme. The work presented in this paper was originally published as a WRC Technical Report.[3]

Organisation of the Work

A Committee was formed to plan the collaborative work, and has representatives† from the Department of the Environment, Scottish Development Department, each Regional

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† The names of representatives at the time the work reported here was carried out are given in the Appendix.

Water Authority (RWA), Scottish River Purification Boards and the WRC. This Committee decided to adopt the approach to the AQC described elsewhere in this issue,[2] each determinand being studied in two phases.

Phase (*i*). One laboratory in each of the ten RWAs and one in Scotland participated, the WRC acting as the co-ordinating laboratory[2]; eleven laboratories were generally involved.

Phase (*ii*). After satisfactory results have been obtained in *Phase* (*i*), those laboratories act as co-ordinators of tests within RWAs and Scotland. Certain RWAs are not involved in this phase because all analyses for Harmonised Monitoring are made by one laboratory.

This paper deals only with *Phase* (*i*).

Required Analytical Accuracy

The following requirements were agreed by the Committee to represent the targets at which to aim[2]; maximum tolerable bias, 10% of the chloride concentration or 0.5 mg l^{-1} of chloride, whichever is the greater; and maximum tolerable total standard deviation, 5% of the chloride concentration or 0.25 mg l^{-1} of chloride, whichever is the greater.

Analytical Quality Control

The approach followed was exactly as presented previously[2]; no attempt is made here, therefore, to explain the reasons underlying the various activities described below. The South West Water Authority was unable to participate in the tests reported here though it has now undertaken the work. The participating laboratories were: Anglian Water Authority, Welland and Nene River Division Laboratory, Oundle; Northumbrian Water Authority, Headquarters Laboratory, Gosforth; North West Water Authority, Mersey and Weaver River Unit Laboratory, Warrington; Severn–Trent Water Authority, Regional Laboratory, Finham; Southern Water Authority, Resource Planning Laboratory, Winchester; Thames Water Authority, Thames Conservancy Division Laboratory, Reading; Welsh Water Authority, Dee and Clwyd River Division Laboratory, Chester; Wessex Water Authority, Bristol Avon Divisional Laboratory, Saltford; Yorkshire Water Authority, Headquarters Laboratory, Leeds; and Forth River Purification Board, Headquarters Laboratory, Edinburgh. The sequence of participating laboratories in the above list does not relate to the order of numbering of laboratories in the Tables.

Choice of Analytical Methods

Participating laboratories each chose a method they thought capable of achieving the required accuracy. The methods involved were (*i*) manual Mohr titration,[4] (*ii*) manual mercury(II) nitrate - diphenylcarbazone titration[4] and (*iii*) semi-automatic continuous flow spectrophotometric methods based on the procedure in reference 5 [mercury(II) thiocyanate - iron(III) salt].

One of the laboratories initially selected a commercially available instrument for the coulometric titration of chloride. Preliminary tests showed, however, that the discrimination with which the instrument response could be read did not allow achievement of the required precision at low concentrations (below 10 mg l^{-1}). This procedure was, therefore, abandoned, and the laboratory adopted method (*iii*).

Within-laboratory Precision and Recovery Tests

Following any preliminary tests considered necessary by the laboratories, each then carried out the same programme of tests to assess the precision of, and certain sources of bias in, its results. Laboratories using method (*iii*) checked that their calibration graphs were linear. All laboratories, on each of 10 days, carried out in random order a batch of analyses consisting of two blank determinations and two portions of each of the following solutions, two standard solutions, a river water and the same river water after addition of a known amount of chloride. Each laboratory collected its own sample of river water from a local source and the same sample was used throughout the tests. The amount of chloride added to the spiked sample varied from laboratory to laboratory, but was in the range 0.2–0.5 C_u, where C_u is the upper concentration limit of a laboratory's method. Each laboratory

prepared its own standard solutions from its own stock standard solution just before each batch of analyses. The concentrations of the two standards used for the tests were usually 0.1 C_u and 0.9 C_u.

On completion of the tests, each laboratory analysed its results statistically to obtain estimates of the within-batch (s_w), between-batch (s_b) and total (s_t) standard deviations,[6] where $s_t = \sqrt{(s_w^2 + s_b^2)}$. The values of s_t for the two standards, the river water and the spiked river water were compared with the appropriate target value using the *F*-test,[2] and were accepted as satisfactory provided s_t was not significantly greater ($p = 0.05$) than the target.[2] The results from these tests are summarised in Table I. For the particular solutions used the chloride concentrations were such that the target for precision was a relative total standard deviation not greater than 5%. This value was exceeded in only three instances, Laboratories 1 and 7 river water and Laboratory 6 standard solution (20 mg l^{-1} of chloride). However, in those instances the observed relative standard deviations were only marginally, and not significantly, greater than 5%. The results were, therefore, considered acceptable.

It is further of interest to note that estimates of precision varied considerably between laboratories, even for the analysis of standard solutions of similar concentration. Of course some of these differences reflect random uncertainty in the estimates of precision. However, some differences between estimates are sufficiently large to indicate real differences in the precision of results obtained by individual laboratories. This reflects differences in the choice of method used and its application in individual laboratories. However, these differences were not important in the context of this exercise and, it is emphasised again, results were within the targets set other than the exceptions discussed above.

Each laboratory also calculated the recovery, *R*, of chloride from the spiked river water, where $R = 100\ (\bar{C}_S - \bar{C}_R)/A$, and $\bar{C}_S$ and $\bar{C}_R$ are the mean concentrations found for the spiked and unspiked river water, respectively, and *A* is the equivalent concentration of chloride added to the spiked sample, allowance being made for the slight dilution of the sample caused by the addition of a standard solution of chloride. It was agreed that recoveries would be considered acceptable if *R* was not significantly worse (*t*-test, $p = 0.05$) than the closer of the two values, 95 and 105%. These results are also summarised in Table I, which shows recoveries between 95 and 104% with an over-all mean of 99.6%. None of the individual recoveries were significantly worse than 95 or 105% and the results were, therefore, considered acceptable.

The satisfactory completion of these tests in all laboratories indicated that within-laboratory precision was adequate and the next stage of the AQC work was started.

Accuracy of Standard Solutions

To ensure that differences in the chloride concentrations of laboratories' standard solutions did not cause important between-laboratory bias, the following tests were made. The WRC prepared a standard solution of sodium chloride (1000 mg l^{-1} of chloride) and portions were sent to all laboratories. Each laboratory then accurately diluted portions of the WRC solution and its own standard solution to nominally the same concentration, which was usually at or near C_u so as to achieve the smallest relative standard deviation. Sufficient replicate analyses of both the diluted standards were made in one batch of analyses so that if there were a difference of 2% in their true concentrations, a statistically significant difference (95% confidence level) between the two means would be found. The required number of analyses on each standard was calculated statistically using the estimates of within-batch standard deviation obtained in the preceding stage of the work.[2]

On completion of the tests, each laboratory compared the mean values obtained for each standard (*t*-test, $p = 0.05$) and the results are summarised in Table II. None of the laboratories' standards differed from the WRC by as much as 2%, the maximum observed difference was 0.4% and the over-all mean difference was 0.1%. These results were considered satisfactory.

Each laboratory then set up a preliminary statistical quality control chart[2] based on the analysis of a standard solution in each subsequent batch of analyses. These charts are intended to aid the continuing, long-term assessment of accuracy in each laboratory and are not further discussed here.

TABLE I

RESULTS FROM WITHIN-LABORATORY PRECISION TESTS

Sample	Parameter	Laboratory No. and analytical method*									
		1, M	2, M	3, MN	4, MN	5, SA	6, SA	7, SA	8, SA	9, SA	10, SA
Standard solution 1	Chloride concentration/mg l^{-1}	25	50	20	8	20	20	30	5	5	20
	Relative total standard deviation,† %	2.6	(0.0)	1.5	1.5	3.6	6.0	3.7	3.8	3.4	4.2
	Total standard deviation†/mg l^{-1}	0.65	(0.0)	0.29	0.12	0.71	1.2	1.1	0.19	0.17	0.84
Standard solution 2	Chloride concentration/mg l^{-1}	200	250	180	80	180	180	270	45	45	180
	Relative total standard deviation,† %	0.26	0.20	0.23	0.18	0.89	1.2	1.0	0.33	0.93	0.89
	Total standard deviation†/mg l^{-1}	0.52	0.49	0.42	0.14	1.6	2.1	2.7	0.15	0.42	1.6
River water	Chloride concentration/mg l^{-1}	6.1	54	47	16	42	40	18	26	23	53
	Relative total standard deviation,† %	5.1	1.3	1.2	0.57	2.2	2.2	5.4	0.92	3.1	2.9
	Total standard deviation†/mg l^{-1}	0.31	0.68	0.55	0.09	0.94	0.89	0.97	0.24	0.71	1.5
Spiked river water	Chloride concentration/mg l^{-1}	106	99	139	65	124	137	111	45	36	144
	Relative total standard deviation,† %	0.36	0.59	0.50	0.11	1.9	1.2	1.4	0.42	2.8	0.96
	Total standard deviation†/mg l^{-1}	0.38	0.58	0.70	0.07	2.4	1.6	1.5	0.19	1.0	1.4
	Mean recovery of chloride from spiked river water,‡ %	100.0 ± 0.1	101.5 ± 1.1	99.2 ± 0.5	99.8 ± 0.1	94.7 ± 1.9	104.2 ± 1.0	98.6 ± 1.0	96.4 ± 1.2	102.6 ± 3.1	99.1 ± 0.8

* M = Mohr titration[4]; MN = mercury(II) nitrate titration[4]; SA = semi-automatic spectrophotometric method based on reference 5 (see Choice of Analytical Methods).
† The total standard deviations have between 9 and 19 effective degrees of freedom.[1]
‡ 95% confidence limits of the mean recovery are also given.

TABLE II

COMPARISON OF PARTICIPANTS' AND WRC STANDARD SOLUTIONS

Laboratory	Mean difference between laboratory and WRC standards,* %
1	−0.16 ± 0.05
2	−0.05 ± 0.29
3	0.33 ± 0.96
4	0.35 ± 0.17
5	0.14 ± 0.99
6	0.09 ± 0.83
7	0.30 ± 0.57
8	0.07 ± 1.00
9	0.07 ± 0.58
10	0.00 ± 0.55
Mean	0.11

* The 95% confidence limits of the mean difference are also given.

Tests of Between-laboratory Bias

To complete this initial phase of AQC, direct checks of any between-laboratory bias were made as follows. The WRC prepared and distributed portions of a standard solution (46.0 mg l^{-1} of chloride) and samples of two different river waters to all laboratories, each participant being given only a broad indication of the concentration of these solutions. One of the river samples was a hard, lowland water, and the other was of the soft, moorland type. Each laboratory analysed each solution once on each of 5 days, and then calculated the mean of the five results and its 90% confidence limits (obtained from the five results only) for each solution. These results were then returned to the WRC for examination for any evidence of bias.

For each solution, tests were made to decide if the mean result from any laboratory could be regarded as a statistical outlier.[7] No such outliers were indicated ($p = 0.05$) for the two river waters. For the standard solution, the result from Laboratory 5 just achieved significance, but neglect of this result only changed the over-all mean from 46.0 to 45.7 mg l^{-1} of chloride. In view of this small difference and because no reasons for regarding the result as suspect were known, it was thought better not to reject the result of Laboratory 5.

The results in Table III show that the confidence intervals of the mean results of different laboratories for a given solution do not all overlap, *i.e.*, a certain amount of between-laboratory bias exists. To assess whether or not the bias of any laboratory exceeded the target value of 10%, the following procedure was used.[2] Denoting the mean and its 90% confidence limits for a given solution and the *i*th laboratory by $\bar{x}_i \pm h_i$, the upper limit (95% confidence) for the bias of the laboratory, U, was calculated as follows: if $\bar{x}_i > T$,

$$U = \frac{100\,(\bar{x}_i + h_i - T)}{T}$$

or, if $\bar{x}_i < T$,

$$U = \frac{100\,(\bar{x}_i - h_i - T)}{T}$$

where T is the true concentration of chloride in the standard solution or the over-all mean concentration of chloride in the river waters. For this purpose, the over-all means for the river waters were calculated from the means of individual laboratories, no weighting for the precisions of the means being used. The values for U are also given in Table III, which shows that they were usually substantially less than the maximum tolerable bias of 10%. In only two instances did U exceed the target and then only marginally (Laboratories 6 and 8 for river water B). However, the mean results of these two laboratories were within 5% of the over-all mean for that sample and their values of U for the other two solutions were much smaller than 10%. It was decided, therefore, that the results from all laboratories could be regarded as satisfactory.

TABLE III

RESULTS FROM TESTS OF BETWEEN-LABORATORY BIAS

Sample	Parameter	Laboratory No. and analytical method* 1, M	2, M	3, MN	4, MN	5, SA	6, SA	7, SA	8, SA	9, SA	10, SA	Mean of all laboratories
Standard chloride solution, 46.0 mg l⁻¹	Mean chloride content†/mg l⁻¹	46.0 ± 0.1	45.0 ± 0.3	46.0 ± 0.4	45.9 ± 0.1	48.0 ± 0.9	46.0 ± 1.2	45.8 ± 1.6	45.0 ± 0.2	46.1 ± 0.4	46.2 ± 0.4	46.0
	Upper limit for bias,‡ %	±0.2	−2.7	±0.9	−0.5	+6.3	±2.7	−3.8	−2.6	+1.2	+1.4	—
River water A ..	Mean chloride content†/mg l⁻¹	97.9 ± 0.2	97.4 ± 0.5	97.0 ± 0.3	96.7 ± 0.2	94.6 ± 1.0	95.7 ± 1.5	97.2 ± 1.6	95.2 ± 0.3	97.1 ± 0.2	§	96.5¶
	Upper limit for bias,‡ %	+1.6	+1.4	+0.8	+0.4	−3.0	−2.4	+2.4	−1.6	+0.8	§	—
River water B ..	Mean chloride content†/mg l⁻¹	8.52 ± 0.17	8.28 ± 0.13	8.80 ± 0.23	8.39 ± 0.11	8.90 ± 0.40	8.24 ± 0.60	8.60 ± 0.52	9.02 ± 0.43	8.48 ± 0.38	8.60 ± 0.52	8.58¶
	Upper limit for bias,‡ %	−2.7	−5.0	+5.2	−3.5	+8.4	−11.0	+6.2	+10.1	−5.6	+6.3	—
	Mean upper limit for bias for the three above solutions, %	−0.4	−2.1	+2.3	−1.2	+3.9	−5.4	+1.6	+2.0	−1.2	+3.9	—

* M = Mohr titration[4]; MN = mercury(II) nitrate titration[4]; SA = semi-automatic spectrophotometric method based on reference 5 (see Choice of Analytical Methods).
† The 90% confidence limits are also given for each mean.
‡ See text for method of calculating the upper limit (95% confidence) for bias.
§ This laboratory originally conducted the work on another semi-automatic system and repeated all the tests on changing to the present system. Insufficient sample of river water A remained for tests with the new system.
¶ The values obtained by the WRC for river waters A and B were 96.2 and 8.48 mg l⁻¹ of chloride, respectively.

At this point the objectives of the initial AQC programme had been achieved, and a similar programme was started for the next determinand of interest, ammoniacal nitrogen.

Routine AQC

To attempt to ensure that the required accuracy of results for chloride is maintained, AQC is now an integral part of the routine analyses for the Harmonised Monitoring Scheme. As mentioned above, prime reliance for this purpose is placed on within-laboratory AQC using statistical quality control charts. However, to obtain direct checks of between-laboratory bias, portions of a river-water sample are distributed occasionally by the WRC to all laboratories. The first four such tests (over a period of approximately 2 years) are of value in indicating the efficiency of the AQC work, and are, therefore, summarised in Table IV. Each of these tests was carried out as described in the previous section except that, for the fourth test, the five replicate analyses were all made in one batch of analyses.

Table IV shows that the upper limit (95% confidence level) for bias is usually less than 10% and that the mean of the individual upper limits is less than 10% for each laboratory. Overall, therefore, it appears that reasonably satisfactory accuracy has been maintained. However, the need for continuing care and emphasis on AQC is indicated by the fact that certain laboratories appear to be rather prone to bias close to or slightly exceeding the target. Therefore, of the 40 sample - laboratory combinations, eight provide upper limits for bias of greater than 10%; of these eight instances, Laboratory 10 gave three, and Laboratories 5 and 9 each gave two.

Each laboratory calculated the standard deviation from the five results for each sample. Of the first three tests (for which the relative total standard deviation could be calculated), no laboratory obtained any values significantly greater ($p = 0.05$) than the target of 5% and only four of the values were greater than 5%. It appears, therefore, that precision was maintained reasonably well.

TABLE IV

RESULTS FROM ROUTINE TESTS OF BETWEEN-LABORATORY BIAS

Laboratory	River water C (September 1975)		River water D (April 1976)		River water E (March 1977)		River water F (December 1977)		
	Mean chloride content/ mg l^{-1}	Upper limit for bias, %	Mean chloride content/ mg l^{-1}	Upper limit for bias, %	Mean chloride content mg l^{-1}	Upper limit for bias, %	Mean chloride content/ mg l^{-1}	Upper limit for bias, %	Mean upper limit for bias, %
1	17.5	+ 3.5	58.0	+ 2.6	29.8	+ 5.3	37.4	+2.5	+3.5
2	18.1	+ 5.7	56.8	− 1.2	29.4	+ 3.3	37.1	+1.7	+2.4
3	17.6	+ 3.9	56.2	− 2.8	27.4	− 6.3	34.6	−7.2	−3.1
4	16.5	− 4.9	56.0	− 2.1	29.0	+ 1.6	35.6	−3.5	−2.2
5	15.6	−14.4	59.0	+ 4.2	26.0	−12.5	34.0*	−7.3	−7.5
6	15.4	−15.5	54.4	− 8.5	28.8	+ 5.8	37.0*	+0.9	−4.3
7	17.4	+ 4.1	56.6	− 3.3	28.8	+ 3.4	40.0*	+9.1	+3.3
8	16.8	− 6.4	57.0	+ 0.2	29.6	+ 3.6	36.0*	−1.8	−1.2
9	18.7	+11.1	58.8	+ 3.9	31.6	+12.2	37.0*	+0.9	+7.0
10	18.6	+11.0	65.0	+16.8	26.4	−16.9	36.8	+1.5	+3.1
Mean†	17.2	—	57.0	—	28.7	—	36.7	—	—

* The five results were reported as identical.

† No laboratory's results were rejected in calculating the over-all mean for samples C, E and F; the result of Laboratory 10 was rejected for sample D.

Discussion

The detailed results provide evidence on a number of points relevant to the design of AQC schemes for a group of laboratories, *e.g.*, the dependence of standard deviation on the concentration of the determinand and the relative importance of different sources of error. However, discussion of such aspects is best deferred until the results for other determinands and analytical techniques have been published. One point is worth a brief mention here, namely the suitability of the accuracy targets for chloride.

The targets for systematic and random errors imply that the tolerable total error (95% confidence level) of individual analytical results is 20% of the chloride concentration or 1 mg l^{-1} of chloride, whichever is the greater. The former target may well be thought rather lax, particularly as chloride is usually regarded as a determinand for which good accuracy is readily achieved. It is interesting, therefore, to consider the consequences of

reducing the maximum tolerable errors to half the values used in this work. If the experimental estimates of errors in this paper were compared with these reduced targets in the manner described above, the following targets would not be achieved: precision tests from Laboratories 1, 6 and 7; recovery tests from Laboratories 5 and 6; accuracy of standard solutions from Laboratories 3, 5 and 8; and between-laboratory bias from Laboratories 3, 5, 6, 7, 8, 9 and 10.

In contrast, with the targets adopted in this work, only two instances occurred where the targets were exceeded, *i.e.*, Laboratories 6 and 8 in the tests of between-laboratory bias. This analysis suggests that substantially greater effort would be required in most laboratories to achieve the smaller targets routinely. Hence, the idea that better accuracy than that aimed for in this work can readily be achieved would seem to be incorrect even for chloride, which is normally regarded as being determined with good accuracy and precision.

The approach to AQC described in this paper aims progressively to identify and, if necessary, control particular sources of error so that a permanently sound basis is established for routine achievement of the required accuracy.[2] The individual tests are, therefore, designed to provide many opportunities for unsuspectedly large errors to reveal themselves. Of all the tests described above, none gave positive evidence that a target was not achieved, although there were several instances where the results indicated that a target could have been exceeded, *e.g.*, the bias of Laboratories 6 and 8 for river water B could have exceeded the target of 10% (see Table III). Such uncertainties are bound to arise as a result of random errors, and in this situation, a partially subjective judgement on the basis of the complete set of tests is necessary. In this way, it is considered reasonable to conclude that all laboratories achieved the required accuracy in the preliminary AQC work. The results of subsequent tests (see Table IV) indicate that this accuracy has been maintained over a period of almost 3 years. So far as is known, this is the first time that such an achievement has been reported for the analysis of river waters.

The success of the work is attributed to two main factors: (*i*) the suitability of the analytical methods adopted by the laboratories; and (*ii*) the sequential approach followed in the AQC programme. The latter involves a relatively large amount of work in each laboratory and a rather long period is necessary to complete all tests. These are disadvantages in the approach, but they are counterbalanced by its ability to ensure that the required accuracy is achieved. Most of the participating laboratories had no previous experience of this type of work, but all agreed that the approach should be retained for tests on other determinands and it is hoped to report the results in subsequent papers.

Conclusions

The targets chosen for accuracy and precision of results appear to be suitable for the Harmonised Monitoring Scheme and capable of achievement for the river waters tested.

All participants achieved the required accuracy during the tests. To ensure that the position is maintained, continuing care is needed, and subsequent analytical quality control will, in addition to normal precautions, be based mainly on the use of quality-control charts and the analysis of samples distributed at regular intervals by the WRC.

The work has confirmed that each of three types of method [*i.e.*, (*i*), (*ii*) and (*iii*) in the section Choice of Analytical Methods] are capable of achieving the target accuracy.

Valuable experience of this collaborative work has been gained. The participating laboratories have commented favourably on the approach adopted and on the co-ordination of the work by the WRC. This approach is now being applied to successive studies of other determinands, the results for which will be reported in subsequent papers.

For those Authorities with more than one participating laboratory, the tests within each area (*Phase ii*) need to be completed before the over-all situation can be regarded as satisfactory. The results in this paper suggest that no undue problems are to be expected in this second stage of testing.

The work has demonstrated a procedure for ensuring permanent comparability of results from many laboratories. Already some of the Authorities have taken advantage of this by extending the procedure to laboratories not directly concerned with the Harmonised Monitoring Scheme. Such extensions are recommended.

ห้องสมุด กรมวิทยาศาสตร์บริการ

Appendix

The following were members of the Analytical Quality Control (Harmonised Monitoring) Committee at the time of the initial work on chloride: Mr. A. L. Wilson, Chairman (Water Research Centre), Dr. E. A. Simpson, Secretary (Department of the Environment), Mr. M. J. Beard (Southern Water Authority), Mr. J. R. Borland (Welsh Water Authority), Mr. R. V. Cheeseman (Water Research Centre), Mr. N. Croft (Yorkshire Water Authority), Dr. B. T. Croll (Anglian Water Authority), Mr. D. V. Hopkin (Thames Water Authority), Mr. J. G. Jones (Wessex Water Authority), Mr. P. Kingslan (Department of the Environment), Mr. J. C. Lambie (Scottish Development Department), Mr. B. Milford (South West Water Authority), Mr. P. Morries (North West Water Authority), Mr. B. D. Ravenscroft (Northumbrian Water Authority), Mr. D. Rodda (Water Data Unit), Mr. J. E. Saunders (Welsh Office), Dr. K. C. Wheatstone (Severn–Trent Water Authority), and Mr. T. Williamson (Forth River Purification Board).

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Received *September 11th*, 1978
Accepted *November 2nd*, 1978

Analyst, April, 1979, Vol. 104, pp. 299–312

Statistical Appraisal of Interference Effects in the Determination of Trace Elements by Atomic-absorption Spectrophotometry in Applied Geochemistry

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Interference effects in the determination of trace elements by atomic-absorption spectrophotometry in applied geochemistry have been studied by a statistical appraisal and described in terms of a simple two-parameter mathematical model. The experimental design incorporated features that would allow possible deviations from the model to be detected, but no serious deviations were detected except in a deliberately chosen example. The study led to the identification of some important interference effects that could affect interpretation of geochemical data and also provided a formula that could be applied to correct the crude results.

Keywords: *Applied geochemistry; mineral exploration; atomic-absorption spectrophotometry; interferences; chemometrics*

Flame atomic-absorption spectrophotometry is by far the most frequently used analytical method in applied geochemistry, whether in the field of mineral exploration or of environmental studies. Although sample media and dissolution techniques vary, most commonly a rock, soil or sediment sample is treated with a mineral acid or mixture of acids to release into solution the trace elements of interest, notably copper, lead, zinc and nickel.[1,2] Depending on the mineralogy of the sample and the acids used, considerable concentrations of the major metallic constituents of such samples are present in the solution presented for analysis. These elements (calcium, magnesium, iron, aluminium, sodium and potassium) are known to cause a variety of interferences, but apart from background correction by means of a continuum source, the effects are largely ignored in applied geochemical work.[3,4] This is because, to be cost-effective, geochemical analyses have to be simple, rapid and cheap. Procedures such as solvent extraction, which separate the trace elements of interest from the major constituents, are usually inadmissible for this reason. Matrix matching is not usually practicable because wide variation in bulk composition within a batch of samples is common.

In view of this widespread use of atomic-absorption spectrophotometry in applied geochemistry it is desirable to have a comprehensive and readily applicable account of the interference effects, so that circumstances where the interference is intolerable (*i.e.*, likely to produce an incorrect interpretation of the data) can be identified.

Govett and Whitehead[5] have studied the effects of the major constituents on the determination of trace amounts of copper, zinc, cobalt and nickel. At low concentrations of the major elements, it was found that the main effects were enhancements due to background absorption. At higher concentrations the enhancements were diminished or even reversed. The effects were found to depend on the concentrations both of the major elements and of the trace elements. However, no clear conclusion was drawn (except the need for caution), because of the difficulty of generalising the results obtained in the absence of any simple mathematical model.

A further difficulty in interference studies is the possibility of complex interactions between the major constituents, producing effects on the apparent concentration of the trace analyte that are not predictable from results obtained with each major constituent separately. This difficulty has been pointed out by Woodis *et al.*[6] and Thompson *et al.*[7] Usually the possibility of complex interactions is ignored because of the difficulty in elucidating them. A simple way to detect such interactions for two interfering elements would be to obtain a three-dimensional response surface at a fixed concentration of the trace analyte, representing

the apparent level of the element as a function of the two interferents. This procedure would require at least a 10 × 10 matrix of points to obtain a reasonable representation of the surface, and the experiment would have to be repeated at several analyte concentrations. However, this approach would scarcely be possible for more than two interferents because of the large number of possible combinations and the difficulty of representing a surface in more than three dimensions.

In this study, we used a simple mathematical model of interference, which greatly facilitated the interpretation of the results obtained and led to straightforward decisions as to whether interference was likely to be important. The model is the same as one that has been widely and successfully used in X-ray fluorescence analysis,[8] but has not, apparently, been fully developed in atomic-absorption work. An experimental design, based on the model, was devised to enable the results to be obtained with the minimum of effort, to allow the data to be tested for possible deviations from the mathematical model and, for the elements studied, to confirm the absence of complex interactions. Where an interference effect was found to be important, the coefficients derived from the data provided correction factors that could be used to improve considerably the accuracy of the raw result.

While our conclusions would be expected to have broad generality in geochemical work, the magnitude of the effects are liable to vary with different instrumental arrangements and operating conditions. Consequently, the data presented in this work should be regarded as illustrating the method of investigation.

Theoretical Model

Interference of a major constituent on a trace analyte can be considered to consist of two components, one independent of the trace analyte concentration and the other dependent on it. In terms of the effect on a trace element calibration graph, these can be considered as a translational effect and a rotational effect as shown in Fig. 1. The translational effect

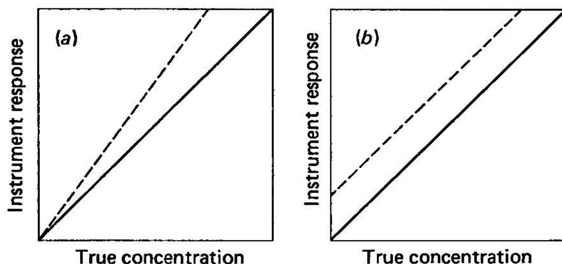

Fig. 1. Results of (*a*) the rotational effect and (*b*) the translational effect on a calibration line. The full lines indicate no interference and the broken lines a constant concentration of interferent.

corresponds, in atomic-absorption spectrophotometry, to "background" interferences, such as molecular absorption and light scattering, and the rotational effects to physical, chemical and ionisation interferences. The two components can operate in combination. In general, the extent of the effect will be a function of the concentration (X) of the major constituent, so that the rotational effect can be formulated as

$$C_A = C_T\, f(X) \quad \ldots \quad (1)$$

where C_A and C_T are, respectively, the apparent concentration and the true concentration of the analyte. A simple assumption is that C_A/C_T is a linear function of X. In this instance $f(0) = 1$, as $C_A = C_T$ when $X = 0$, and we have

$$C_A = C_T\,(1 + aX) \quad \ldots \quad (2)$$

where a is the constant coefficient for the rotational effect.

In a similar way, the translational effect can be formulated as

$$C_A = C_T + f(X) \quad \ldots \quad (3)$$

Again, the assumption is made that the effect is a linear function of X. In this instance $f(0) = 0$ and we obtain the equation

$$C_A = C_T + bX \quad \ldots \quad (4)$$

where b is the coefficient for the translational effect. When the two effects are operating together we have

$$C_A = C_T(1 + aX) + bX \quad \ldots \quad (5)$$

or

$$C_A = C_T + d_C X \quad \ldots \quad (6)$$

where $d_C = C_T a + b$.

Experimental quantification of a and b can be undertaken in the following manner. For a given value of C_T, determine the value of d_C [$= (dC_A/dX)_{C_T}$] by plotting the apparent concentration at various levels of X. The slope will have a constant value if the initial assumptions are correct. This is illustrated in Fig. 2. In a similar manner values of d_C at various levels of C_T are obtained. As $d_C = C_T a + b$, a plot of d_C against C_T will give a line with a slope of a and intercept of b, as illustrated in Fig. 3. With the values of a and b determined, for known X the true concentration can be obtained from the apparent by inverting equation (5):

$$C_T = (C_A - bX)/(1 + aX) \quad \ldots \quad (7)$$

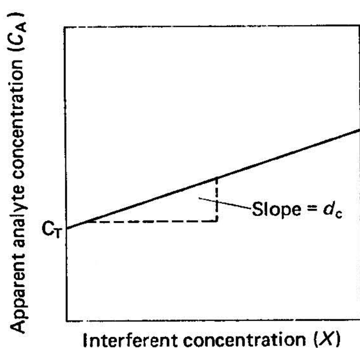

Fig. 2. Effect of increasing interferent concentration (X) on the apparent analyte concentration (C_A) for a fixed level of analyte (C_T).

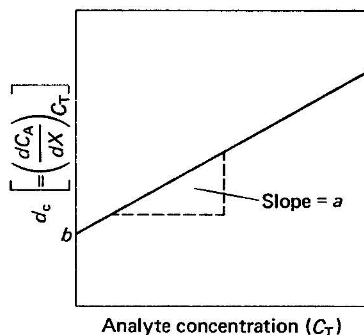

Fig. 3. Relationship between d_C and C_T, showing how to obtain values of a and b.

If more than one interfering major constituent is present and complex interactions do not occur, the interference can be represented by the equation

$$C_A = C_T(1 + \Sigma a_i X_i) + \Sigma b_i X_i \quad \ldots \quad (8)$$

where a_i, b_i and X_i refer to values for the ith interfering element, and all the effects are independent and additive. In that instance the individual a_i and b_i can be obtained as

previously, by studying the effect on C_A of varying each X_i separately, or by allowing all of the X_i to vary simultaneously, separating the effects by means of multiple regression techniques.

There are no *a priori* reasons for believing that the simplifying assumptions (of independent, linear, additive effects) are correct. The experimental design must therefore contain features that enable these assumptions to be tested. If the system under study conforms fairly closely to the model, as demonstrated in the results presented here, the interference effects can be characterised simply and quantified quickly. If there are serious deviations, a much more extensive programme of work may be called for.

Experimental

Apparatus

All measurements were made on a Perkin-Elmer 403 atomic-absorption spectrophotometer. Readings were made without background correction in the concentration mode, with linearisation where appropriate. Gas flow-rates were those recommended in the instrument handbook, the response being optimised for flame height and nebuliser adjustment. Instrumental conditions are shown in Table I. Single element standards were prepared in a 1 M hydrochloric acid solution.

TABLE I

INSTRUMENTAL CONDITIONS

Element	Wavelength/nm	Lamp current/mA	Slit width
Cadmium	228.8	10	4
Cobalt	240.7	20	3
Copper	324.7	15	4
Lithium	335.4	15	4
Manganese	279.5	20	3
Nickel	232.0	20	3
Lead	283.3	10	4
Titanium	365.3	25	3
Zinc..	213.8	20	4

Reagents

The trace metal solutions were prepared from BDH atomic-absorption standards, except titanium, which was prepared from potassium titanium oxalate. The major element solutions were prepared from specially purified samples of the metal chlorides. De-ionised water and AnalaR hydrochloric acid were used throughout.

Computing

All computing was carried out on Imperial College Computer Centre's CDC 6400/7314 facility. Multiple regression was performed by a slightly modified version of the routine STEPR from the IBM Scientific Subroutine Package. Regression with analysis of variance for lack of fit, and weighted linear regression were carried out, respectively, by means of the FORTRAN routines REPELF and WAYLIN written by one of the authors (MT).

Experimental Design

One hundred solutions were prepared in 1 M hydrochloric acid, each of which contained the trace analytes (*viz.*, cadmium, cobalt, copper, lithium, manganese, nickel, lead and zinc) and the major constituents (*viz.*, aluminium, calcium, iron, potassium, magnesium and sodium) at a pre-determined level. The concentration levels selected for the analytes spanned the useful calibration range for each element, while those of the major constituents covered the range likely to be encountered in solutions derived from rocks, soils and sediments. The concentrations are shown in Tables II and III. The 100 solutions were divided into five sets of 20. Within each set all of the trace analytes were present at only one of the five concentration levels, the five sets thus covering the whole range of the trace concentrations. For each solution within a set, the concentration of each major element was

TABLE II

CONCENTRATIONS OF ANALYTES ($\mu g\ ml^{-1}$) USED IN INTERFERENCE STUDY

Analyte	Level 1	2	3	4	5
Cadmium	0.00	0.48	0.88	1.43	1.90
Cobalt	0.00	1.19	2.19	3.57	4.76
Copper	0.00	1.19	2.19	3.57	4.76
Lithium	0.00	1.19	2.19	3.57	4.76
Manganese	0.00	2.38	4.38	7.14	9.52
Nickel	0.00	1.19	2.19	3.57	4.76
Lead	0.00	2.38	4.38	7.14	9.52
Zinc	0.00	0.48	0.88	1.43	1.90

individually selected at random from its ten possible levels in such a way that each level was selected exactly twice. An example of such a randomised scheme is shown in Table IV. The apparent concentration of trace analytes in each solution was then determined by atomic-absorption spectrophotometry, each solution being analysed twice in a random sequence.

TABLE III

CONCENTRATIONS OF INTERFERENTS (% *m*/*V*) USED IN INTERFERENCE STUDY

Interferent	Level 1	2	3	4	5	6	7	8	9	10
Aluminium	0	0.0190	0.0380	0.0570	0.0760	0.0952	0.1143	0.1333	0.1524	0.1905
Calcium	0	0.0952	0.1905	0.2857	0.3810	0.4762	0.5714	0.6667	0.7619	0.9524
Iron	0	0.0190	0.0380	0.0570	0.0760	0.0952	0.1143	0.1333	0.1524	0.1905
Potassium	0	0.0190	0.0380	0.0570	0.0760	0.0952	0.1143	0.1333	0.1524	0.1905
Magnesium	0	0.0190	0.0380	0.0570	0.0760	0.0952	0.1143	0.1333	0.1524	0.1905
Sodium	0	0.0190	0.0380	0.0570	0.0760	0.0952	0.1143	0.1333	0.1524	0.1905

The types of sample preparation generally used in applied geochemical work do not lead to the presence of silicon in solution except sometimes as a trace constituent, and silicon was therefore not included among the interferents. Other non-metallic interferents (*e.g.*, sulphur and phosphorus) are likely to be present only in minor concentrations compared with the metals, and were excluded from this study. The solutions used are thus reasonably representative of those derived from real samples.

The randomisation scheme is necessary in order to avoid any systematic effects, which are thereby converted into random effects. In addition, complex interactions may be obscured if there is any correlation between the concentrations of the major elements. The duplication of major element concentrations is required in order to make a statistical test for lack of fit (caused in this instance by non-linearity). The duplication of the measurements is undertaken to obtain an estimate of instrumental variance, to provide a base-level against which other variances can be compared. The experimental design does not allow for the possibility of interference from trace element interactions. These are assumed to be unimportant at such concentrations.

Results and Discussion

The essential feature in the interpretation of the data is the analysis of variance at each level of a trace analyte. If there were no interference then each solution would produce an identical result (apart from instrumental and volumetric variations). In fact, all of the solutions give different results owing to the interferences and this is quantified by the total variance. The analysis of variance distributes this total variance between (*i*) that which can be accounted for by linear relationships with the major constituent concentrations, *i.e.*, by linear regressions; (*ii*) that due to "lack of fit," *i.e.*, by a failure of the initial

TABLE IV

EXAMPLE OF A RANDOMISED DUPLICATED SCHEME FOR THE CONCENTRATIONS OF THE MAJOR ELEMENTS

Solution number	Concentration level*					
	Aluminium	Calcium	Iron	Potassium	Magnesium	Sodium
1	1	4	7	6	8	1
2	8	8	10	2	3	4
3	3	6	8	3	5	9
4	2	7	8	1	7	3
5	6	5	1	7	2	7
6	6	10	2	2	4	5
7	8	4	6	1	9	3
8	4	3	6	6	10	6
9	1	1	1	4	3	8
10	9	2	3	8	2	1
11	7	6	7	3	5	9
12	9	5	9	4	6	8
13	7	2	4	10	1	7
14	2	8	5	7	7	6
15	5	3	10	8	8	5
16	4	9	9	5	9	2
17	10	7	2	5	6	2
18	3	10	5	10	1	4
19	10	9	3	9	4	10
20	5	1	4	9	10	10

* For actual concentrations corresponding to these levels, see Table III.

assumption that the interference effects are linear functions of the major element concentrations; (*iii*) that due to complex interactions, *i.e.*, by a failure of the second assumption; and (*iv*) that due to instrumental variations and volumetric errors. Each of these features is illustrated in the ensuing discussion. The diagrams are not usually necessary for the statistical analysis but serve here to illustrate several of its features, and are sometimes helpful in interpreting dubious examples. A comprehensive account of regression methods is given by Draper and Smith.[9]

Copper

The first stage of the procedure is the multiple regression, which is carried out separately for each level of the trace analytes. Thus, for copper at level 1 (*i.e.*, zero concentration) STEPR produced the results summarised in Table V. The regressions are given in decreasing order of significance as shown by the values of Student's *t*. Only the regressions on calcium and iron are significant at the 95% confidence limits. The intercept (0.002) is not significantly different from the true value of zero. The standard error of the estimate is equivalent to the standard deviation of the distances between the experimental points and the calculated

TABLE V

MULTIPLE REGRESSION OF COPPER ON MAJOR CONSTITUENTS

Results at level 1 (zero analyte concentration).

Major constituent	Regression coefficient	Standard error of coefficient	*t*-value
Calcium	0.1569	0.0090	17.36
Iron	0.1088	0.0473	2.30
Aluminium	0.0294	0.0434	0.68
Potassium	0.0272	0.0457	0.60
Magnesium	0.0109	0.0484	0.23
Sodium	0.0091	0.0437	0.21

Intercept = 0.0020.
Standard error of estimate = 0.013.
Explained variance = 96.5%.

lines. In this instance it is not significantly greater than normal instrumental noise levels (0.01 $\mu g\ ml^{-1}$) as judged from the duplicated measurements. The implication is that all of the measurable interference can be attributed to calcium and iron by linear relationships with their concentrations. However, consideration of the standard error of the estimate is not a certain test of good regression, as will be shown below, and other statistics should be considered at the same time. In the context of this work, the percentage variance explained and an examination of residuals are useful supplementary tests.

The multiple regression is repeated at each of the analyte concentration levels. The next stage of the procedure is illustrated by the results obtained for the interference effects of calcium on copper, but the same procedure is followed for each analyte - interferent combination. The regression data extracted for the copper - calcium pair are given in Table VI.

TABLE VI

STATISTICS FOR THE REGRESSION OF APPARENT CONCENTRATION OF COPPER ON CALCIUM CONCENTRATION, AT VARIOUS CONCENTRATIONS OF COPPER

Analyte level	Copper concentration/ $\mu g\ ml^{-1}$	Regression coefficient	Standard error of coefficient	*t*-value	Standard error of estimate*
1	0.00	0.1569	0.0090	17.36	0.013
2	1.19	0.0429	0.0172	2.50	0.024
3	2.19	−0.0138	0.0181	−0.76	0.025
4	3.57	−0.0897	0.0309	−2.91	0.043
5	4.76	−0.1454	0.0462	−3.14	0.065

* This value is the residual after all the regressions are included, not only calcium.

These data and statistics are illustrated in Fig. 4, which shows the apparent concentrations of copper as a function of calcium concentration. (It must be noted that the visible differences between the duplicated results at each level of calcium is not variance caused by analytical error but by the different levels of all of the other major elements.) Both the data and Fig. 4 show the regression coefficient changing from positive to negative as the copper concentration increases. At approximately 2 $\mu g\ ml^{-1}$ of copper the coefficient is zero, showing that at this concentration there is no apparent effect of calcium on copper,

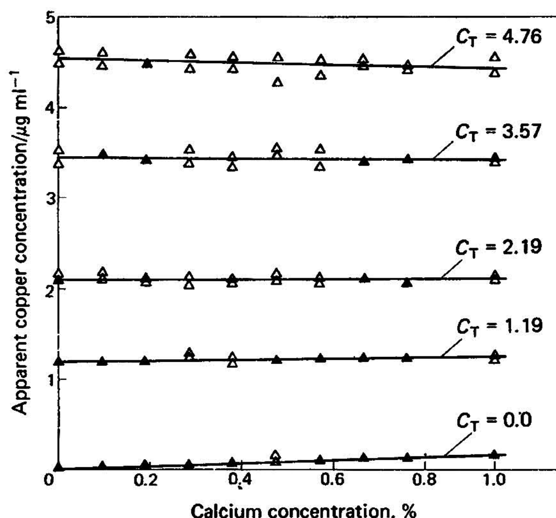

Fig. 4. Effect of calcium concentration on the apparent concentration of copper at various true concentrations (C_T) of the analyte. Open points, single observations; and closed points, two coincident observations. This system shows a significant change in slope with C_T.

even though there is an effect at both higher and lower copper concentrations. This highlights the erroneous conclusions that can be drawn from interference studies carried out at a single trace element concentration. The standard error of the estimate is satisfactorily low at each copper concentration level, suggesting that there is no measurable non-linearity or complex interactions for copper.

The values of the regression coefficients correspond to variable d_C in equation (6) while the analyte concentrations correspond to C_T. The values of a and b can now be obtained by regression of d_C on C_T. It is necessary to obtain also the standard errors of the intercept (b) and the slope (a) so that the values can be tested as to whether they are significantly greater than zero. However, normal regression can give misleading results for the standard errors, as the variance at higher trace levels, necessarily greater than at lower levels, has too much influence on the regression. Weighted regression therefore has to be used. In this technique, each observation is weighted by a factor inversely proportional to its variance. The variance in this instance is the square of the standard error of the coefficient. The necessity for this procedure is illustrated in Fig. 5. The error bars on each point represent the 95% confidence limits, *i.e.*, twice the standard error. It is clear that the regression lines produced by weighted regression and by simple regression are very close and not significantly different. However, the standard errors of the intercepts are very different. The 95% confidence limits for the simple regression include zero, implying that the intercept is not significant. The same confidence limits for the weighted regression, which are clearly more realistic, show the intercept to be highly significant.

The values extracted for copper were $a = -0.071$ ($t = 11.2$) and $b = 0.150$ ($t = 16.1$). Hence, the measurable interference from calcium on copper under the conditions studied can be expressed by $C_T = (C_A - 0.150X)/(1 - 0.071X)$ where C_T and C_A are expressed in micrograms per millilitre and X as a percentage.

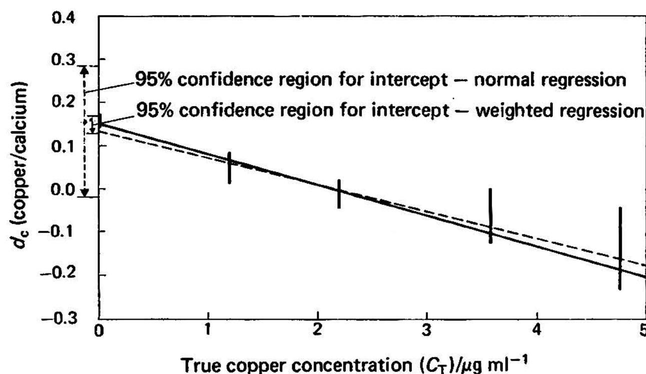

Fig. 5. Regression of d_C on C_T for copper/calcium. The error bars are the 95% confidence regions for the d_C values. Broken line, normal regression; and solid line, weighted regression.

The Other Analytes

The procedure outlined for copper was repeated for the other seven analytes. The results are summarised in Table VII, which shows all of the a and b values that were more significant than 95%. Most of the values were also more significant than 99% and those are shown in italic type. All but two of the a values were negative, representing suppression of the analytical signal by the major component. The exceptions were sodium and potassium on lithium. Most of the b values are positive, representing positive changes in background absorption, which in most instances accounts for the greater part of the interference. Broadly, the effects found were as expected from experience of atomic-absorption spectrometry, in confirmation of the adequacy of the model. An example of an analyte - interferent pair (cadmium - calcium), which shows only background interference, is illustrated in Fig. 6. As before, non-adherence to the theoretical model was tested by examination of the standard errors of the estimate, and the percentage variance explained, at each level for each analyte.

TABLE VII

SIGNIFICANT VALUES OF *a* AND *b* FOUND IN THE INTERFERENCE STUDY

Figures in italic type are significant at 99% level, others at 95% level. Asterisks indicate "important" effects.

Major constituent		Trace analyte							
		Cd	Co	Cu	Li	Mn	Ni	Pb	Zn
Aluminium ..	*a*	−0.114	_−0.320*_	_−0.168_	_−0.224*_	_−0.162_	_−0.290*_		_−0.111_
	b		_1.026*_		_0.044_				_0.068_
Calcium	*a*		−0.051	_−0.071_	_−0.262*_	_−0.066_		_−0.062_	
	b	_0.213*_	_0.852*_	_0.150*_	_0.036_	_0.193_	_1.277*_	_1.601*_	_0.133_
Iron	*a*				_−0.281_				
	b				_−0.016_			_0.831_	_0.360_
Potassium ..	*a*				0.065				
	b								_0.114_
Magnesium ..	*a*			−0.111	_−0.118_				
	b						_−0.315_		
Sodium	*a*				0.019				
	b	0.085	0.547		_−0.011_		_0.559_		

Of the analytes only lithium showed significantly large standard errors. Non-linear effects accounted for the deviation in the lithium results. These effects were tested for by performing a regression with analysis of variance for "pure error" and "lack of fit" at each analyte level. In this technique, the variance caused by the difference between the pairs of results for each level of the interferent (which is due to the effect of different concentrations of the other major elements) is compared with that related to the mean distance of the two points from the regression line. This procedure is illustrated in Fig. 7, which shows the results of the regression of the lithium results on calcium. The individual results are plotted together with the calculated linear regression lines. For the higher concentrations of lithium the diagram clearly shows a non-linear effect, which the linear regression underestimates in some ranges and overestimates elsewhere. All of the significant regressions were tested

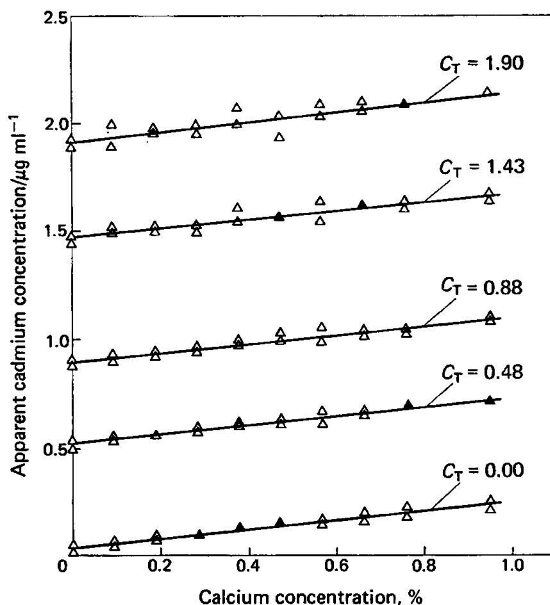

Fig. 6. Effect of calcium on the apparent concentration of cadmium at various levels. This system shows only "background" interference. Symbols as in Fig. 4.

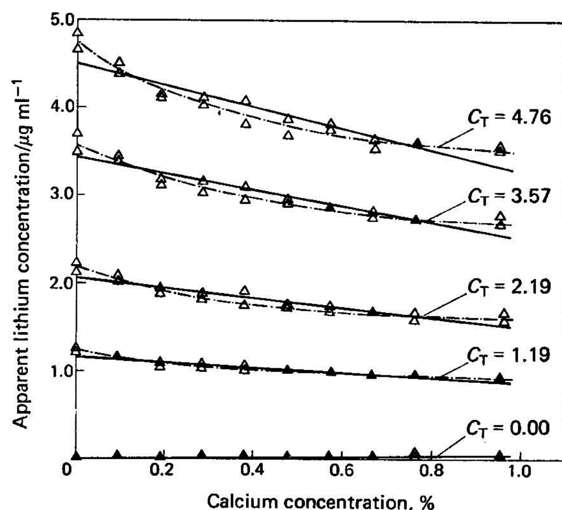

Fig. 7. Effect of calcium concentration on the apparent concentration of lithium at various levels. This shows the significant lack of fit of the linear regressions (solid lines) at the higher levels. Symbols as in Fig. 4.

individually in this way, but only lithium (at four levels) and cobalt (at one level) were found to have significant lack of fit, as judged by the value of the F (variance ratio) test. To obtain an adequate fit for such data a more elaborate mathematical relationship is required. Generally, polynomial fits do not give satisfactory results. However, it is clear from Fig. 7 that even a linear fit would provide a substantial improvement in accuracy. For cobalt, inspection of the residuals strongly suggested that the isolated significant lack of fit was caused by a random fluctuation rather than a non-linear effect at one level. The statistical test alone cannot distinguish between the two possibilities.

The success of the two-parameter model in describing the interferences was tested by applying to the experimental data corrections based on the inverse of equation (8), *i.e.*,

$$C_T = (C_A - \Sigma b_i X_i)/(1 + \Sigma a_i X_i) \quad \quad .. \quad .. \quad .. \quad (9)$$

TABLE VIII

CORRECTION OF EXPERIMENTAL RESULTS BY USE OF TWO-PARAMETER MODEL

The results of all significant corrections have been applied to the uncorrected (raw) data to give the corrected value (corr.) for comparison with the true value. Means are given for each level (standard deviations in parentheses).

Trace level	Result	Analyte concentration/µg ml⁻¹							
		Cadmium	Cobalt	Copper	Lithium	Manganese	Nickel	Lead	Zinc
1	Raw	0.12 (0.07)	0.63 (0.27)	0.09 (0.05)	0.02 (0.01)	0.11 (0.07)	0.83 (0.42)	1.02 (0.49)	0.12 (0.05)
	Corr.	0.03 (0.01)	0.17 (0.09)	0.02 (0.01)	0.00 (0.00)	0.03 (0.04)	0.25 (0.09)	0.25 (0.09)	0.01 (0.01)
	True	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	Raw	0.61 (0.06)	1.85 (0.26)	1.21 (0.03)	1.03 (0.09)	2.43 (0.05)	1.96 (0.39)	3.42 (0.44)	0.59 (0.04)
	Corr.	0.52 (0.01)	1.43 (0.10)	1.20 (0.02)	1.23 (0.02)	2.42 (0.05)	1.42 (0.08)	2.71 (0.09)	0.49 (0.01)
	True	0.48	1.19	1.19	1.19	2.38	1.19	2.38	0.48
3	Raw	0.98 (0.06)	2.79 (0.25)	2.09 (0.03)	1.82 (0.18)	4.22 (0.06)	2.87 (0.40)	5.28 (0.41)	0.95 (0.05)
	Corr.	0.89 (0.01)	2.40 (0.10)	2.13 (0.03)	2.17 (0.06)	4.25 (0.06)	2.35 (0.09)	4.63 (0.08)	0.85 (0.02)
	True	0.88	2.19	2.19	2.19	4.38	2.19	4.38	0.88
4	Raw	1.56 (0.06)	4.15 (0.23)	3.42 (0.05)	3.02 (0.29)	6.99 (0.12)	4.30 (0.35)	8.18 (0.36)	1.55 (0.05)
	Corr.	1.46 (0.02)	3.79 (0.17)	3.52 (0.04)	3.62 (0.06)	7.11 (0.08)	3.81 (0.11)	7.61 (0.10)	1.45 (0.03)
	True	1.43	3.57	3.57	3.57	7.14	3.57	7.14	1.43
5	Raw	2.01 (0.07)	5.32 (0.23)	4.48 (0.09)	3.97 (0.39)	9.17 (0.21)	5.43 (0.38)	10.47 (0.37)	2.02 (0.05)
	Corr.	1.92 (0.03)	5.00 (0.16)	4.62 (0.06)	4.76 (0.11)	9.35 (0.17)	4.98 (0.14)	9.97 (0.17)	1.93 (0.02)
	True	1.90	4.76	4.76	4.76	9.52	4.76	9.52	1.90

for all significant values of *a* and *b*. The results obtained are summarised in Table VIII, which shows, for each level of each trace analyte, the mean and standard deviation of all the solutions of the uncorrected (raw) results, the corrected results and the true concentration present. In virtually all instances the mean result was brought substantially closer to the true result and the range (as indicated by the standard deviation) was markedly reduced, showing a considerable degree of success with the two-parameter model. Some of the data are illustrated in Fig. 8 for two different types of interference.

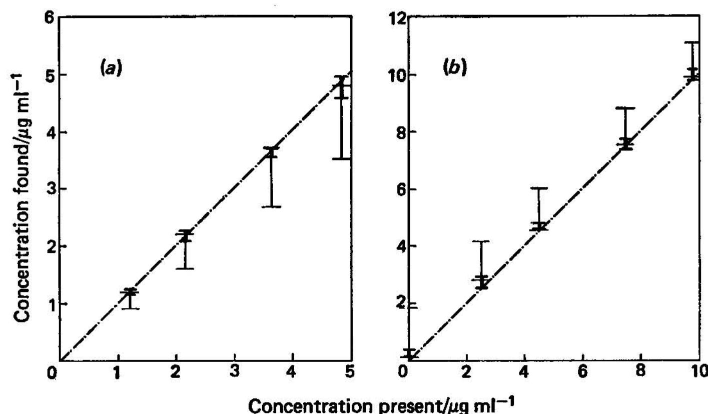

Fig. 8. Range of experimental results for (*a*) lithium and (*b*) lead, both uncorrected (narrow bars) and corrected with all significant parameters (thick bars).

The Magnitude of the Interferences

While the procedure described above enabled all of the statistically significant (*i.e.*, measurable) effects to be quantified, it did not in itself provide information as to whether the magnitude of the effects was likely to be "important" (*i.e.*, likely to affect interpretation of geochemical data). This information can be obtained by the application of empirical criteria appropriate to the data usage. For geochemical work we have applied the following criteria: an effect is "important" if $|a| > 0.05/M_{99}$ or $|b| > T_5/M_{99}$, where M_{99} is the 99th percentile concentration (in per cent.) of the major element in the population of samples to be analysed, and T_5 is the fifth percentile of the trace element concentration (in micrograms per millilitre). Thus, when the factor *a* is equal to the criterion, only for one sample in 100 will the interference effect exceed a relative change of $\pm 5\%$ in the apparent analyte concentration. For lesser major concentrations a smaller interference will be found. When *b* is equal to the criterion, the interference will equal the analyte concentration only when the interferent exceeds its 99th percentile at the same time as the trace analyte falls below its 5th percentile, *i.e.*, a probability of 0.0005 if the concentrations of the two elements are independent. The "importance" of the *b* value thus depends on the concentration range of the analyte. For example, the *b* value for cadmium - calcium of 0.213 is graded "important": a sample of pure limestone (which contains 40% calcium) would produce an apparent cadmium level of 40×0.213 or about 8 p.p.m. compared with the normal level of <1 p.p.m. This is clearly important. The corresponding effect for zinc would amount to 40×0.133 or 5.3 p.p.m. However, the normal level of zinc is in the range 50–100 p.p.m. so the effect is not important.

The "important" effects identified in this way are shown in Table VII with an asterisk. The percentiles were taken from a major geochemical survey involving 50000 samples.[9] Only aluminium and calcium produce "important" effects in the context of geochemical analysis. Aluminium produces mainly rotational type effects (factor *a*), on cobalt, lithium and nickel. Calcium produces translational effects (factor *b*) on cadmium, cobalt, copper, nickel and lead. While the background interference can be at least partly corrected by means of absorption measurements with a continuum source, there is no universally applicable method for removing rotational effects.

An Example of Complex Interaction

The interference effects studied could be effectively accounted for by a simple model of linear, independent, additive effects. Only in one instance (calcium on lithium) was a significant non-linear effect identified, and even here the linear approximation was an adequate model. In these circumstances the methodological approach suggested in this work is capable of elucidating the interference effects. However, it is often suspected and sometimes demonstrated that more complex types of interference are occurring. We have examined one such system, the interference of iron and aluminium on titanium, which is known to have non-linear effects and complex interactions.[11,12] The sole purpose of this additional study was to examine the performance of the statistical approach under conditions where the simple assumptions of the method are likely to fail, and to determine whether the failure manifests itself clearly by the statistical tests. Titanium would be only partially extracted by the acid attacks described here. The experimental design was as described above, with titanium at 5 levels (0, 10, 20, 30 and 40 μg ml^{-1}) and iron and aluminium within the ranges 0–10000 and 0–2000 μg ml^{-1}, respectively, and with no other interferent present.

Multiple regression (the first stage of the data analysis) produced the statistics summarised in Table IX. The percentages of the variances explained were small, the standard errors of the estimates were large compared with the pure analytical error, and the regression coefficients and values of t were small. In addition, analysis of variance for lack of fit (titanium against aluminium) showed highly significant lack of fit. Thus, in all these ways the statistics show a non-conforming interference system. Only the low regression coefficients might suggest to the unwary that there was no significant interference. The reason for this is the extreme non-linearity of the effect of aluminium. This is illustrated in Fig. 9, which shows the failure of linear regression to represent this type of data. Thus, it is important to take note of all statistics and visually to examine the residual plots in doubtful instances.

A two-way plot of these data showed that there were complex interactions in the system as well as non-linear features. The data produced in the simple experiment were not sufficient to characterise the response surface and a more comprehensive experiment was required. It was found that the effect of iron and aluminium can be represented approximately by the surface shown in Fig. 10, for any level of titanium. It is difficult adequately to express a complex surface like this in terms of polynomial trend analysis.

TABLE IX

STATISTICS FOR THE MULTIPLE REGRESSION STUDIES ON THE INTERFERENCE OF ALUMINIUM AND IRON ON TITANIUM

Analyte level	Interferent	Regression coefficient	t-value	Standard error of estimate	Variance explained, %
1	Iron	0.000	0.004	0.74	18.5
	Aluminium	0.005	2.89		
2	Iron	0.001	1.20	1.81	37.5
	Aluminium	0.002	0.37		
3	Iron	0.000	0.07	2.8	18.9
	Aluminium	−0.002	−0.25		
4	Iron	0.005	2.55	3.8	15.1
	Aluminium	−0.004	−0.42		
5	Iron	−0.008	−3.82	3.8	30.0
	Aluminium	0.000	−0.06		

Summary and Conclusions

It has been shown that a simple two-parameter model of interference with independent, linear, additive effects is adequate to describe the measurable interference in the atomic-absorption determination of cadmium, cobalt, copper, lithium, manganese, nickel, lead and zinc in geochemical samples. The statistically significant parameters have been evaluated

and those of important magnitude have been indicated by an empirical but uniformly applicable criterion. This enables the analyst to identify conditions where interferences might have a noticeable effect on data interpretation, and where appropriate, to make suitable corrections in the form of equation (9). Where large numbers of data are involved the corrections can be applied automatically by computer. This will become increasingly easy as microprocessor-controlled data logging systems become more common.

Although an elaborate experimental design and data appraisal are required in order to characterise the interference model, the parameters a and b can be evaluated with only four solutions once it has been established that the system conforms to the model. Naturally the values of a and b will depend on the instrumental settings, the use of background correction and other factors, and should be checked for each analytical batch. In addition, it is good practice to reduce interferences to as low a level as possible before attempting corrections, which should generally be regarded as a last resort.

Where deviations from the model occur, the experimental design ensures that they are detected and suggests how more elaborate tests can be made to characterise them. In most of these instances informal designs in interference studies would give misleading results, especially with regard to complex interactions in multi-interferent systems, which usually remain undetected. These effects may still remain too complex to elucidate fully, but even so, it is important for the analyst to know when these occur. Where they occur it is necessary to resort to the use of appropriate standard solutions whose major constituents correspond in composition to those of the average sample.

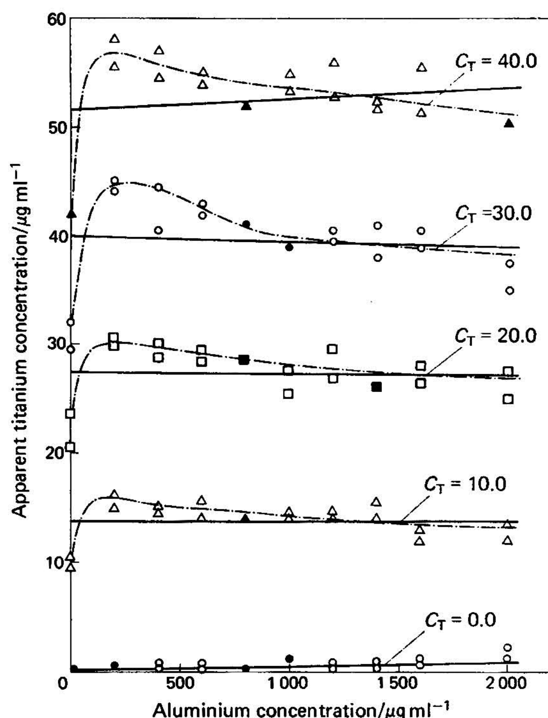

Fig. 9. Effect of aluminium on the apparent concentration of titanium in the system Ti - Al - Fe. This shows failure of the linear system to represent these data. Symbols as in Fig. 4.

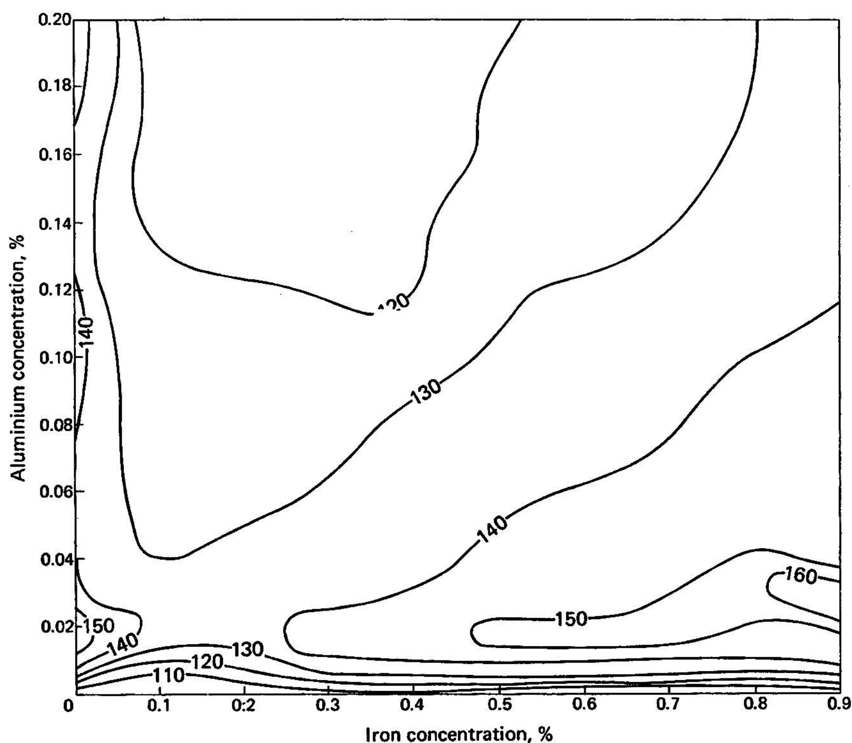

Fig. 10. Combined effect of aluminium and iron on the apparent concentration of titanium at 30 μg ml^{-1}. The contours indicate relative enhancement as a percentage.

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Received *June 26th*, 1978
Accepted *November 15th*, 1978

Analyst, April, 1979, Vol. 104, pp. 313–322

Minimum Sample Preparation for the Determination of Ten Elements in Pig Faeces and Feeds by Atomic-absorption Spectrophotometry and a Spectrophotometric Procedure for Total Phosphorus

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Studies of mineral metabolism in pigs and problems of manure disposal or utilisation are complicated by interactions of trace metals and major cations. A procedure for the determination of copper, zinc, cadmium, lead, iron, sodium, potassium, magnesium, calcium, phosphorus and arsenic in pig faeces and feeds is described. Phosphorus is determined spectrophotometrically and the other elements by atomic-absorption spectrophotometry. Sample preparation is minimised, and all elements except arsenic are determined after a single sample digestion in nitric acid - perchloric acid mixture. A separate sample digestion is necessary for arsenic. The accuracy and precision of the method were rigorously tested, and are suitable for budget studies of all eleven elements.

Keywords: *Pig faeces and feed analysis; trace metal determination; major element determination; atomic-absorption spectrophotometry; spectrophotometry*

Intensification in animal production in recent years has generated problems in relation to the disposal or utilisation of the large amounts of manure produced per annum. Manures from pig, poultry or dairy/beef units have traditionally been spread on land to utilise their fertiliser values. With the trend towards larger intensive units, and a decrease in land area suitable for manure spreading from such units, attention is being given to alternative means of manure disposal. The environmental effects of disposing of large amounts of manure to small land areas is causing concern, particularly where the manures contain high concentrations of metals.[1,2] Commercially formulated pig diets generally contain supplemental mineral salts to improve growth and development and to compensate for deficiencies due to interactions between some elements. Minerals at high levels in the feed are not fully absorbed by the pig and can be concentrated by a factor of four in the faeces.[2] Because of the unusually high levels of minerals in pig faeces and feeds, these materials provide an unusual matrix when the accurate determination of element concentrations is required.

Investigations into the fate of minerals or trace elements in biological systems are complicated by the interactions of some elements with each other, and with other metabolites in the system.[3,4] For this reason, it is rare to encounter experimental work on mineral metabolism that reports the functions and fate of single elements without examining the influence of potential interacting elements within the system under investigation. Hence, it is essential that laboratories use methods that permit determination of the maximum number of elements but with the minimum of sample preparation. Our investigations were principally aimed at the development of economical methods for the analysis of pig manures and feeds for a wide range of elements, giving results of high accuracy and precision. To minimise sample manipulation, calibration over a wide range was used and linear or quadratic equations were fitted to the calibration lines. Concentrations of elements in the

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sample solutions were computed using these equations. Some degree of burner rotation was used for the more sensitive and abundant elements.

Experimental

Apparatus

Atomic-absorption spectrophotometer. A Varian Techtron, Model 1200, instrument with an air - acetylene burner and digital read-out was used for the determination of copper, zinc, cadmium, lead, iron, sodium, potassium, magnesium and calcium. A Varian Techtron, Model AA5, instrument with a BC-6 simultaneous background corrector and a Mace 1100 strip-chart recorder was used for the determination of arsenic. A Varian Techtron, Model 64, hydride-generation kit was used with a nitrogen - hydrogen flame using the burner supplied for air - propane.

Spectrophotometer. A Unicam SP600 instrument with a 1-cm light path flow-through cell attachment was used for the determination of phosphorus.

Computation. A Sharp, Model 365P, programmable calculator was used to fit linear calibration lines to the equation $y = a + bx$ and quadratic curvilinear calibration lines to the equation $y = a + bx + cx^2$.

Kjeldahl digestion flasks. Flasks of capacity 50 ml on an electric heat bank with individual heat controls were used.

Glass culture tubes with PTFE seal screw-caps. Capacity 25 ml.

Reagents

All solutions were prepared using glass-distilled water in glassware that had previously been washed with 10% nitric acid and rinsed with distilled water. Concentrated acids from Ajax Chemicals Pty. Ltd. were selected batches of low trace-element content. Unless specified otherwise, all chemicals used were of analytical-reagent grade.

Nitric acid, sp. gr. 1.42.

Dilute nitric acid, 2 M. Dilute 127 ml of the concentrated acid to 1000 ml with water.

Perchloric acid, 70%, *sp. gr.* 1.54.

Sulphuric acid, sp. gr. 1.84.

Hydrochloric acid, sp. gr. 1.18.

Nitric acid - perchloric acid digestion mixture. Mix equal volumes of the concentrated acids.

Sulphuric acid - perchloric acid - sodium molybdate digestion mixture. Dissolve 2 g of sodium molybdate ($Na_2MoO_4.2H_2O$) in 40 ml of water, add 50 ml of concentrated sulphuric acid, allow the mixture to cool and add 10 ml of concentrated perchloric acid.

Ammonia solution, sp. gr. 0.880.

Bromophenol blue indicator solution, 0.1% *m/V in methanol.*

Citrate buffer. Dissolve 210 g of citric acid and 29.5 g of trisodium citrate in water and dilute to 1000 ml with water.

Ammonium tetramethylenedithiocarbamate solution, 0.5% *m/V in water.* Prepare fresh daily.

4-Methylpentan-2-one. Purified by extraction with 2 M nitric acid.

Sodium tetrahydroborate(III) solution, 5% *m/V in* 0.1% *sodium hydroxide solution.* Use within 1½ h of preparation.

Lanthanum - caesium solution, 5% *m/V lanthanum and* 2% *m/V caesium in water.* Dissolve 2.5 g of caesium chloride in 100 ml of water and add 100 ml of 10% *m/V* lanthanum solution (BDH standard solution for spectroscopy) .

Vanadate reagent for phosphate determination. Solution A: dissolve 25 g of ammonium molybdate in 400 ml of water. Solution B: dissolve 1.25 g of ammonium metavanadate in 300 ml of boiling water, cool and add 200 ml of concentrated perchloric acid. Add solution A to solution B and dilute to 1000 ml with water.

Arsenic standard solutions. Dissolve 0.8329 g of sodium arsenate ($Na_2HAsO_4.7H_2O$) in water and dilute to 1000 ml with water. Dilute 3 ml of this solution to 200 ml with water to give a 3 μg ml^{-1} arsenic stock standard solution. Prepare arsenic standard solutions in the range 0.03–0.36 μg ml^{-1} by diluting appropriate volumes of the stock standard solution with 5% sulphuric acid.

Cadmium standard solutions. BDH cadmium standard solution for atomic-absorption spectrophotometry, containing 1000 $\mu g\ ml^{-1}$ of cadmium, diluted with 2 M nitric acid to give standard solutions in the range 0.05–1.50 $\mu g\ ml^{-1}$ of cadmium. These solutions should be saturated with 4-methylpentan-2-one prior to use.

Lead standard solutions. BDH lead standard solution for atomic-absorption spectrophotometry, containing 1000 $\mu g\ ml^{-1}$ of lead, diluted with 2 M nitric acid to give standard solutions in the range 0.5–15.0 $\mu g\ ml^{-1}$ of lead. These solutions should be saturated with 4-methylpentan-2-one prior to use.

Multi-element stock standard solution. The following stock solutions are prepared for inclusion in the multi-element stock standard solution:

A. 1000 $\mu g\ ml^{-1}$ solutions of copper, zinc and iron; available as BDH standard solutions for atomic-absorption spectrophotometry.
B. 10000 $\mu g\ ml^{-1}$ of calcium; 6.2425 g of calcium carbonate dissolved in 120 ml of water plus 15 ml of concentrated hydrochloric acid. Dilute to 250 ml with water.
C. 10000 $\mu g\ ml^{-1}$ of magnesium; 20.270 g of magnesium sulphate ($MgSO_4.7H_2O$) dissolved in 150 ml of water. Add 2 ml of concentrated hydrochloric acid and dilute to 200 ml with water.
D. 10000 $\mu g\ ml^{-1}$ of sodium; 6.355 g of sodium chloride dissolved in and diluted to 250 ml with water.
E. 100000 $\mu g\ ml^{-1}$ of potassium; 19.08 g of potassium chloride. Add 1 ml of concentrated hydrochloric acid and dilute to 100 ml with water.
F. 10000 $\mu g\ ml^{-1}$ of phosphorus; 10.6603 g of ammonium orthophosphate [$(NH_4)_2HPO_4$] dissolved in and diluted to 250 ml with water.

Appropriate volumes of each stock solution are added to a 1000-ml calibrated flask and diluted to volume with water to give a multi-element stock standard solution containing each element at the following concentrations ($\mu g\ ml^{-1}$):

Cu	Zn	Fe	Na	K	Mg	Cu	P
20	60	100	2000	1000	1000	2000	2000

Procedure for Measuring Accuracy and Precision of Analytical Techniques

Samples of pig faeces were collected from 24 commercial piggeries, freeze-dried and ground through a 1-mm screen of a stainless-steel laboratory mill. Sub-samples from each sample were bulked to provide a large representative sample of pig faeces. The accuracy and precision of the techniques were assessed as follows.

1. The over-all precision of the techniques was determined by analysis of at least six replicate 2-g samples of dried, ground pig faeces.
2. To determine the recovery of each element and assess losses due to volatilisation or precipitation during digestion, known amounts of each element were added to triplicate 2-g samples of the bulk faeces sample before and after digestion. These standard additions were made by adding 10 ml of the multi-element stock standard solution. The arsenic recovery was determined separately by adding 1 ml of the arsenic stock standard solution to triplicate 2-g samples.
3. To establish if sample matrix effects such as ionisation or chemical interference affected the accurate determination of each element, the following procedure was adopted. Prior to digestion, 10- and 15-ml aliquots of the multi-element stock standard solution were added to 1.0-, 1.5- and 2.0-g masses of faeces in triplicate. Effects on arsenic determination were examined separately by adding 1 and 2 ml of the arsenic standard solution to triplicate 1- and 2-g masses of faeces before digestion.
4. The accuracy of the methods was assessed by analysing the US National Bureau of Standards Reference Material 1571, "orchard leaves." Duplicate 2-g samples were digested and analysed by the method described below.

Digestion Procedure

The preparation of biological material for multi-element analysis can be accomplished in several ways. Dry ashing, which requires heating the sample to temperatures above

400 °C, was considered to be unsuitable because of the potential losses of lead, cadmium and arsenic due to volatilisation at the ashing temperatures.[5] Wet digestion, by heating with acids, was considered to be a more suitable means of destroying organic matter. The choice of reagents for wet digestion was made after considering the solubility of salts formed during digestion. Sulphuric acid was not used when the likely losses of calcium and lead as insoluble sulphates would be significant. A mixture of nitric and perchloric acids (1 + 1) was selected as the digestion mixture for copper, zinc, cadmium, lead, iron, sodium, potassium, magnesium, calcium and phosphorus. An oxidising solution of sodium molybdate in sulphuric - perchloric acids was used for arsenic samples.

For determination of copper, zinc, cadmium, lead, iron, sodium, potassium, magnesium, calcium and phosphorus

Approximately 2 g of sample were weighed accurately into Kjeldahl flasks and digested initially with 15 ml of concentrated nitric acid (sp. gr. 1.42) and finally with 15 ml of the nitric - perchloric acid digestion mixture. Appropriate additions of the multi-element standard solution were made before or after digestion as required. The digest solutions were then transferred quantitatively into 100-ml calibrated flasks and diluted to the mark with water.

For determination of arsenic

Approximately 2 g of sample were weighed accurately into Kjeldahl flasks and digested initially with 10 ml of concentrated nitric acid (sp. gr. 1.42) and finally with 15 ml of the sulphuric acid - perchloric acid - molybdate digestion mixture. Standard additions of arsenic were made as required. The digest solutions were then transferred quantitatively into 50-ml calibrated flasks and diluted to the mark with water.

Determination of Elements

The instrument settings and flame conditions suitable for each element are shown in Table I. A 25-ml aliquot of digest solution was removed from each 100-ml calibrated flask

TABLE I

INSTRUMENT SETTINGS AND FLAMES USED FOR ATOMIC-ABSORPTION SPECTROPHOTOMETRY

Condition	Element									
	Cu	Zn	Cd	Pb	Fe	Na	K	Mg	Ca	As
Wavelength (nm)	324.7	213.9	228.8	217.0	372.0	330.2	404.4	285.2	422.7	193.7
Slit width (mm)	0.2	0.2	0.5	1.0	0.5	1.0	1.0	0.5	0.2	0.25
Flame	Air - acetylene									H_2 - N_2

for the determination of copper, zinc, iron, sodium, potassium, magnesium, calcium and phosphorus. The digest solution remaining in the flask (approximately 75 ml) was retained for the determination of cadmium and lead. The multi-element stock standard solution was diluted with 4% perchloric acid to give standards within the ranges shown in Table II.

TABLE II

ELEMENT CONCENTRATIONS IN THE MULTI-ELEMENT STANDARD SOLUTIONS

Element	Lowest standard/ µg ml^{-1}	Highest standard/ µg ml^{-1}	Stock standard/ µg ml^{-1}
Cu	0.4	16	20
Zn	1.2	48	60
Cd	0.004	0.16	0.2
Pb	0.04	1.6	2
Fe	2	80	100
Na	40	1600	2000
K	20	800	1000
Mg	20	800	1000
Ca	40	1600	2000
P	40	1600	2000

Copper, zinc and iron

Copper and iron are generally free from interference during atomic-absorption spectrophotometry, so the digests and standards were nebulised directly into the flame. The copper calibration graph was linear over the complete range and had a correlation coefficient (r_{xy}) of 0.999. Copper concentrations in the sample digests were computed from a linear regression equation. The iron calibration line was curved above 30 μg ml^{-1} and concentrations of iron in sample solutions were computed from a quadratic equation fitting the calibration line. Because non-atomic absorption from sample solutions was considered possible at the zinc wavelength, the absorbance was also measured separately at 213.9 nm using a hydrogen lamp. No significant absorbance was observed using the hydrogen lamp with the necessary 80° burner rotation. The zinc calibration line was linear up to 24 μg ml^{-1} (r_{xy} = 0.998) and was used up to 60 μg ml^{-1} by fitting a quadratic equation to the curve.

Cadmium and lead

Levels of cadmium and lead in faeces and feeds were found to be too low for direct determination on the digest solution. A procedure for the concentration of these elements by solvent extraction was developed utilising ammonium tetramethylenedithiocarbamate and 4-methylpentan-2-one. Optimum conditions for the extraction of trace amounts of cadmium and lead from the digest solution were determined experimentally. The efficiency of the extraction procedure was determined as follows. Appropriate volumes of the multi-element stock standard solution were diluted to 100 ml with 4% perchloric acid to give solutions containing 0.004–0.100 μg ml^{-1} of cadmium and 0.04–1.0 μg ml^{-1} of lead in a multi-element matrix similar to the sample digest. A blank of 100 ml of perchloric acid was also prepared.

A 25-ml volume was removed by pipette from each flask, leaving 75 ml of solution, similar to the sample digest volume available for analysis. Bromophenol blue indicator was added to each flask and ammonia solution (sp. gr. 0.880) added dropwise until the end-point was reached. A 5-ml volume of 1 M citrate buffer (pH 4.0) was added, followed by 3 ml of 0.5% ammonium tetramethylenedithiocarbamate solution. The contents of each flask were mixed, 10 ml of 4-methylpentan-2-one added and the contents mixed again. The organic layer was allowed to separate, then transferred by Pasteur pipette into a 25-ml culture tube. The aqueous phase was extracted with a further 5 ml of 4-methylpentan-2-one, which was also added to the culture tube. As the cadmium and lead complexes are not stable for more than about 2 h,[6] cadmium and lead were back-extracted from the 4-methylpentan-2-one into 5 ml of 2 M nitric acid. This procedure eliminated the necessity of preparing standards in 4-methylpentan-2-one. The upper organic layer was removed from the culture tube, leaving the cadmium and lead in 2 M nitric acid. Complete extraction of cadmium and lead from the standards would yield solutions containing 0.06–1.50 μg ml^{-1} of cadmium and 0.6–15.0 μg ml^{-1} of lead. Standard solutions of cadmium and lead covering similar concentration ranges were prepared in 2 M nitric acid saturated with 4-methylpentan-2-one to simulate the extracted standards.

Absorbances of the extracted and non-extracted standards were measured using the conditions described in Table I. The instrument was adjusted to zero with distilled water and the absorbance of a 2 M nitric acid solution saturated with 4-methylpentan-2-one subtracted from all readings. The absorbance of extracted and non-extracted cadmium and lead standards showed that the recovery of cadmium and lead through the extraction procedure was essentially quantitative (Table III). This extraction procedure was applied to test faeces samples digests.

Magnesium and calcium

To overcome potential problems due to ionisation or chemical interferences in the determination of magnesium and calcium, various combinations of releasing agents and ionisation suppressants were examined under different flame conditions. The most suitable conditions were as follows.

Sample and standard solutions were diluted 1 + 49 by adding 2 ml of a solution of 5% lanthanum - 2% caesium to 0.2 ml of sample or standard and diluting to 10 ml with water. These solutions were aspirated into the instrument using the settings shown in Table I and gave curved calibration lines over the standard ranges used.

TABLE III

COMPARISON OF ABSORBANCES OF EXTRACTED AND NON-EXTRACTED CADMIUM AND LEAD STANDARD SOLUTIONS, SHOWING RECOVERY BY THE SOLVENT-EXTRACTION PROCEDURE

Cadmium standards				Lead standards			
	Absorbance				Absorbance		
Standard concentration/ $\mu g\ ml^{-1}$	Extracted	Non-extracted	Recovery, %	Standard concentration/ $\mu g\ ml^{-1}$	Extracted	Non-extracted	Recovery, %
0.06	0.010	0.009	111	0.6	0.009	0.010	90
0.15	0.024	0.023	104	1.5	0.019	0.020	95
0.30	0.049	0.047	104	3.0	0.038	0.036	106
0.75	0.120	0.117	103	7.5	0.095	0.092	103
1.50	0.221	0.229	97	15.0	0.186	0.180	103

Sodium and potassium

Sodium and potassium can form refractory compounds during digestion and may also ionise in an air - acetylene flame. To overcome these effects, 4 ml of sample or standard solutions were added to 1 ml of a solution containing 5% lanthanum - 2% caesium before aspirating into the instrument using the settings shown in Table I. The calibration lines were curved over the standard range used.

Phosphorus

The molybdophosphovanadate yellow colour method described by Jackson[7] was examined for its applicability to the determination of phosphorus levels in pig faeces digest solutions. A 1.0-ml volume of sample or standard solution was mixed with 10 ml of molybdovanadate reagent, then diluted with water to 50 ml in a calibrated flask, and the colour was allowed to develop for at least 10 min. Using a wavelength of 440 nm and a 1-cm light path, a linear calibration graph was obtained for standards in the range 2–15 $\mu g\ ml^{-1}$ of phosphorus.

Arsenic

Arsenic can be determined in biological materials by the generation of arsine and measurement by atomic-absorption spectrophotometry,[8] or colorimetrically using silver diethyldithiocarbamate.[9] The atomic-absorption method was chosen because it has the greater sensitivity needed for the materials being studied. Preliminary experiments using digestion with nitric - perchloric acids followed by arsine generation using the method described by Duncan and Parker[8] showed that variable losses of up to 50% occurred during digestion. Double peaks of arsenic absorbance were recorded, indicating that arsine was being formed at two different reaction rates. A similar occurrence was observed by Aggett and Aspell,[10] who used the differences in reaction rates to separate arsenic(III) from arsenic(V) in sample digests. Another digestion technique was sought that would not result in losses of arsenic and that would maintain arsenic in a single valency state for hydride generation.

Simon *et al.*[11] showed that loss of arsenic as the volatile arsenic(III) chloride during acid digestion procedures can be prevented by maintaining strong oxidising conditions by inclusion of sodium molybdate (Na_2MoO_4) in the digestion mixture. Comparison of the recoveries of trivalent and pentavalent arsenic added to samples before and after digestion showed that under their digestion conditions arsenic was converted into and maintained in the non-volatile pentavalent form, and using a coulometric technique recoveries of approximately 94% were obtained. This procedure was adapted for prevention of losses during digestion and double-peak formation with arsine generation.

Test faeces samples were digested for arsenic according to the procedure described earlier. Arsenic standard solutions in the range 0.03–0.36 $\mu g\ ml^{-1}$ in 5% sulphuric acid were prepared from the 3 $\mu g\ ml^{-1}$ arsenic stock standard solution. A 2-ml volume of sample or standard solution was placed in the reaction vessel with 2.5 ml of concentrated hydrochloric acid. Arsine was generated by the addition of 5 ml of 5% sodium tetrahydroborate(III) in 0.1% sodium hydroxide solution and measured using the instrumental conditions shown in Table I. The calibration line of arsenic concentration *versus* peak height was curved over

the standard range and was fitted by a quadratic equation. No significant losses of arsenic occurred during digestion of samples (Table V), and arsine was generated at one reaction rate, as indicated by the absence of double peaks during measurement.

Results and Discussion

The over-all precision of the procedure is summarised in Table IV and shows that coefficients of variation of better than 5% were obtained for all elements except lead and arsenic. The higher variability associated with the determination of these two elements is consistent with their presence at relatively low concentrations in the sample.

TABLE IV

RESULTS OF REPLICATE ANALYSIS OF 2-g SAMPLES OF BULK PIG FAECES USING THE RECOMMENDED PROCEDURE

Parameter	Element										
	Cu	Zn	Cd	Pb	Fe	Na	K	Mg	Ca	P	As
Number of replicates	6	6	12	12	6	6	6	6	6	6	6
Mean concentration (dry matter basis)	290 $\mu g\ g^{-1}$	511 $\mu g\ g^{-1}$	0.86 $\mu g\ g^{-1}$	8.96 $\mu g\ g^{-1}$	1 740 $\mu g\ g^{-1}$	0.32%	0.83%	0.82%	3.88%	2.45%	2.0 $\mu g\ g^{-1}$
Standard deviation	10.5 $\mu g\ g^{-1}$	23.8 $\mu g\ g^{-1}$	0.04 $\mu g\ g^{-1}$	0.84 $\mu g\ g^{-1}$	40 $\mu g\ g^{-1}$	0.01%	0.03%	0.02%	0.09%	0.06%	0.27 $\mu g\ g^{-1}$
Coefficient of variation, %	3.6	4.7	4.8	9.4	2.3	3.3	3.8	2.1	2.4	2.3	13.5

The mean recovery of standard additions of all elements made before and after digestion are summarised in Table V. Application of Student's *t*-test indicated no significant losses of any element during the digestion procedure. This is important as the literature frequently reports losses of cadmium, lead and arsenic in procedures for determining these elements in biological material.

TABLE V

STATISTICAL COMPARISON OF RECOVERIES OF ELEMENTS ADDED TO 2 g OF PIG FAECES BEFORE AND AFTER DIGESTION

Element	Number of replicates	Amount added	Mean recovery, %		Student's *t*-test ($P < 0.05$)
			Pre-digestion addition	Post-digestion addition	
Cu	3	200 μg	105.8 ± 1.5	97.5 ± 9.8	N.S.*
Zn	3	600 μg	98.6 ± 3.6	95.1 ± 6.8	N.S.
Cd	4	2 μg	91.9 ± 4.4	87.2 ± 4.0	N.S.
Pb	4	20 μg	101.7 ± 12.4	93.0 ± 17.8	N.S.
Fe	3	1000 μg	97.9 ± 6.5	93.2 ± 10.1	N.S.
Na	4	20 mg	100.7 ± 2.6	101.9 ± 4.4	N.S.
K	4	10 mg	101.5 ± 11.8	101.9 ± 9.6	N.S.
Mg	3	10 mg	105.2 ± 9.0	100.1 ± 7.1	N.S.
Ca	3	20 mg	93.5 ± 9.6	98.5 ± 12.3	N.S.
P	3	20 mg	90.3 ± 4.4	91.5 ± 9.0	N.S.
As	3	3 μg	91.1 ± 8.5	110.5 ± 9.0	N.S.

* N.S. indicates not significant.

The mean recoveries of all elements added to increasing masses of sample are summarised in Table VI. The recoveries were quantitative for copper, zinc, lead, sodium, potassium, magnesium, calcium and arsenic. Lower recoveries, but better than 90%, were observed for cadmium, iron and phosphorus. These losses could not be accounted for by losses during digestion (Table V) or by chemical association or precipitation with other elements or compounds in the sample material (Tables V and VI). The losses were of constant magnitude and could perhaps be explained by the formation of insoluble salts of the digesting acids or by the absorption of small amounts of these elements on the surface of the digestion flask. Similar losses for iron were reported by the US National Bureau of Standards in the analysis of their reference materials. Coefficients of variation around the mean percentage recovery

of cadmium, lead and iron (Table VI) were sufficiently low to permit the valid application of factors to correct the results obtained with the technique described. Measured concentrations of cadmium, iron and phosphorus (in sample digests) were corrected for apparent losses by dividing by 0.90, 0.92 and 0.94, respectively. These factors represent the mean recovery of these elements as shown in Table VI.

TABLE VI

MEAN PERCENTAGE RECOVERIES OF TWO LEVELS OF STANDARD ADDITION TO INCREASING MASSES OF A REPRESENTATIVE PIG FAECES SAMPLE

Element	Standard addition	Mean* recoveries (%) from			Mean† recovery using pooled data, %
		1.0 g	1.5 g	2.0 g	
Cu	200 μg	100.3	102.6	97.2	101.1 ± 5.3
	300 μg	98.6	104.1	102.5	C.V.‡ = 5.2%
Zn	600 μg	99.8	98.4	104.8	102.1 ± 6.0
	900 μg	106.7	105.7	107.9	C.V. = 5.9%
Cd	2.0 μg	83.7	94.7	89.8	90.1 ± 4.9
	3.0 μg	93.7	89.2	90.8	C.V. = 5.4%
Pb	20 μg	99.3	101.9	96.6	99.5 ± 10.3
	30 μg	108.7	101.0	95.1	C.V. = 10.3%
Fe	1000 μg	93.2	94.2	83.7	92.0 ± 7.1
	1500 μg	90.5	89.8	93.1	C.V.= 7.7%
Na	20 mg	99.1	102.3	99.9	99.6 ± 3.2
	30 mg	95.7	96.6	99.6	C.V. = 3.2%
K	10 mg	95.8	105.0	97.3	100.1 ± 8.0
	15 mg	97.3	102.5	98.4	C.V. = 8.0%
Mg	10 mg	91.7	97.2	102.6	100.5 ± 6.5
	15 mg	102.9	103.4	100.4	C.V. = 6.5%
Ca	20 mg	94.6	98.8	107.7	98.0 ± 7.9
	30 mg	100.9	97.5	92.9	C.V. = 8.0%
P	20 mg	96.5	90.8	94.9	93.9 ± 5.2
	30 mg	100.4	95.4	91.3	C.V. = 5.5%
As	3.0 μg	99.0		91.1	97.0 ± 12.1
	6.0 μg	95.2		94.9	C.V. = 12.5%

* Mean of 3 determinations.
† Mean of 18 determinations, except for As, which is mean of 12 determinations.
‡ C.V. = coefficient of variation.

A two-way analysis of variance was performed to establish if the recovery was affected by two levels of standard addition to increasing masses of sample material, and the results are shown in Table VII. This method of analysis showed an apparently significant effect ($P < 0.05$) for zinc between recovery and level of standard addition. The mean recovery of 600 μg of zinc added to 1.0, 1.5 and 2.0 g of faeces was 101.0 ± 6.2%, whereas the recovery of 900 μg of zinc from similar masses of sample was 106.7 ± 1.7%. Differences of this order could be attributed to pipetting errors and no real practical significance could be attached to these variations. The statistical analysis in Table VII indicated an interactions effect between sample mass, standard addition and recovery for both calcium and cadmium. The recovery data for calcium (Table VI) indicated that when 20 mg of calcium were added to increasing masses of faeces the percentage recovery increased with mass, but when 30 mg of calcium were added the recovery decreased with increasing mass of faeces. These results imply that an optimum range existed for calcium recovery and that the maximum recovery of added calcium was obtained when the total concentration of calcium (from sample and standard addition) in the digest was between 700 and 1000 μg ml^{-1}. It is possible that

TABLE VII

TWO-WAY ANALYSIS OF VARIANCE TO DETERMINE THE EFFECTS ON RECOVERY OF TWO LEVELS OF STANDARD ADDITION TO THREE DIFFERENT MASSES OF BULK PIG FAECES

	Mean square (M.S.) and degrees of freedom (D.F.) for source of variation							
	Increased sample mass		Increased standard addition		Interaction		Error	
Element	M.S.	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.	D.F.
Cu	27.97	2	13.35	1	18.27	2	19.47	12
Zn	28.87	2	145.64*	1	8.41	2	21.42	12
Cd	16.01	2	15.49	1	91.24*	2	17.40	12
Pb	104.39	2	24.50	1	55.77	1	56.89	12
Fe	25.61	2	2.64	1	84.93	2	41.97	12
Na	6.26	2	24.50	1	24.37	2	7.62	12
K	87.32	2	0.02	1	7.48	2	57.64	12
Mg	28.77	2	115.52	1	68.53	2	27.15	12
Ca	11.77	2	48.35	1	171.88*	2	37.88	12
P	57.42	2	11.36	1	31.36	2	19.48	12
As	79.05	1	2.80	1	23.52	1	188.87	4

* Indicates significance at $P < 0.05$.

these results are a statistical or analytical anomaly, as the analysis of NBS orchard leaves 1571 (Table VIII) by our technique gave a calcium level of 2.11%, which is in good agreement with the quoted level of 2.09 ± 0.03%. The digest solution of this material had a calcium concentration of approximately 420 $\mu g\ ml^{-1}$, which is outside the apparent optimum range. The interaction effect on cadmium recoveries (Table VII) is barely significant and there were no obvious trends in recovery associated with increasing sample mass or standard addition. We concluded that any apparent significance of the interaction was fortuitous as at a 5% probability level it is possible to obtain one erroneous result in twenty. Our study involved a total of 33 analyses of variance and it was possible for us to obtain at least one apparently significant result due to chance.

TABLE VIII

ANALYSIS OF NBS ORCHARD LEAVES 1571 USING THE PROPOSED PROCEDURE

Element	Certified concentration	Concentration found*
Cu	12 ± 1 $\mu g\ g^{-1}$	12 $\mu g\ g^{-1}$
Zn	25 ± 3 $\mu g\ g^{-1}$	27 $\mu g\ g^{-1}$
Cd	0.11 ± 0.02 $\mu g\ g^{-1}$	0.09 $\mu g\ g^{-1}$
Pb	45 ± 3 $\mu g\ g^{-1}$	47 $\mu g\ g^{-1}$
Fe	300 ± 20 $\mu g\ g^{-1}$	287 $\mu g\ g^{-1}$
Na	82 ± 6 $\mu g\ g^{-1}$	—†
K	1.47 ± 0.03%	1.48%
Mg	0.62 ± 0.02%	0.61%
Ca	2.09 ± 0.03%	2.11%
P	0.21 ± 0.01%	0.20%
As	14 ± 2 $\mu g\ g^{-1}$	16 $\mu g\ g^{-1}$

* Dry matter basis. Concentrations quoted as found are means of duplicate analyses.

† Na was not determined as its concentration in the reference material fell below the calibration range of our procedure.

Results of analysis of NBS orchard leaves 1571 are shown in Table VIII. The level of sodium in the orchard leaves is outside the working range of the proposed procedure. Sodium levels similar to those found in orchard leaves can be determined by using the more sensitive sodium line at 589.0 nm. Comparison of the certified values for orchard leaves and those found by using our technique (Table VIII) confirmed the accuracy of our methods. All of the concentrations we found were within the standard deviations associated with the certified levels.

Our procedure offers high accuracy and precision when used to measure the concentrations of these eleven elements in pig faeces. It is applicable to other biological materials, as shown by analysis of NBS orchard leaves 1571, and can be used to determine economically the concentrations of copper, zinc, cadmium, lead, iron, sodium, potassium, calcium, magnesium and phosphorus from a single digestion. Arsenic can be determined separately using the acid - molybdate digestion solution.

Application of the Procedure

These procedures were used to analyse samples of pig faeces and pig feeds collected from commercial piggeries in Victoria, Australia. Results of these analyses were used to determine the mineral status of the pig rations and to evaluate the environmental consequences of various methods of disposal or utilisation of piggery effluents. Typical levels of the elements assayed for are presented in Table IX.

TABLE IX

ANALYSIS OF PIG DIETS AND PIG FAECES USING THE PROPOSED PROCEDURE (DRY MATTER BASIS)

Sample	No.	Cu/ $\mu g\ g^{-1}$	Zn/ $\mu g\ g^{-1}$	Cd/ $\mu g\ g^{-1}$	Pb/ $\mu g\ g^{-1}$	Fe/ $\mu g\ g^{-1}$	Na, %	K, %	Mg, %	Ca, %	P, %	As/ $\mu g\ g^{-1}$
Diet	1	23	231	0.02	1.20	299	0.13	0.49	0.16	1.50	1.00	0.5
	2	163	152	0.27	1.24	341	0.25	0.71	0.19	1.65	1.25	0.2
	3	183	167	0.31	1.57	458	0.37	0.74	0.21	1.35	1.26	0.5
	4	20	140	0.04	2.85	353	0.20	0.51	0.11	1.14	0.93	32.4
	5	8	97	0.02	1.22	241	0.14	0.50	0.13	0.68	0.66	0.4
Faeces ..	1	64	766	0.04	12.65	5 098	0.18	0.86	0.48	3.21	1.99	4.4
	2	364	572	0.74	20.73	2 098	0.24	1.05	0.68	4.18	2.04	1.1
	3	569	802	1.20	3.88	6 407	0.20	0.99	0.82	4.63	3.13	19.7
	4	76	622	0.32	6.84	5 306	0.28	0.80	0.51	3.40	3.26	102.6
	5	55	616	0.26	0.29	1 557	0.12	0.87	0.82	2.93	2.15	18.3

The authors thank Mr. G. Perry, Mr. R. Reid and Mr. E. Butler for their assistance during the development and calibration of these analytical techniques.

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Received *August 31st*, 1977
Amended *August 8th*, 1978
Accepted *October 2nd*, 1978

Sulphochlorophenol N as a Spectrophotometric Reagent for Vanadium(V)

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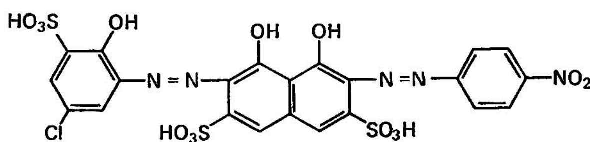
A spectrophotometric method for the determination of trace amounts of vanadium(V) with sulphochlorophenol N is described. With this reagent, vanadium forms a blue complex, which is stable in the pH range 3.7–6.0. The coloured complex obeys Beer's law at 627 nm in aqueous solution with a molar absorptivity of 3.12×10^4 l mol^{-1} cm^{-1}. Copper and cobalt ions interfere in this method.

Keywords: *Bisazochromotropic acid dye*; *spectrophotometry*; *sulphochlorophenol N*; *vanadium determination*

Many bisazochromotropic acid derivatives, such as arsenazo III, are well known as highly sensitive spectrophotometric reagents for various metal ions.[1] Alimarin and co-workers[2,3] reported that some derivatives of 4,5-dihydroxynaphthalene-2,7-disulphonic acid (chromotropic acid) were useful reagents for the photometric determination of niobium and other metals (zirconium, hafnium, vanadium, molybdenum, scandium, aluminium, indium, gallium and palladium). Sulphochlorophenol S {3,6-bis[(5-chloro-2-hydroxy-3-sulphophenyl)azo]-4,5-dihydroxynaphthalene-2,7-disulphonic acid}, which is a symmetrical derivative, has been used for the determination of niobium in steels,[2,4] tantalum metal,[5] titanium oxide,[6] uranium[7] and zirconium alloys.[2] However, no derivatives that could be used for the determination of vanadium, which is in the same Group as niobium in the Periodic Table, have been reported.

Further investigation of bisazo derivatives of chromotropic acid has revealed that the introduction of a nitro group in the *para*-position in one of the benzene rings, such as arsenazo-*p*-NO_2[8,9] or carboxynitrazo,[10] produces a high sensitivity and selectivity and a wide absorption peak shift. Preliminary studies have shown that an asymmetric bisazo derivative, sulphochlorophenol N {3-[(5-chloro-2-hydroxy-3-sulphophenyl)azo]4,5-dihydroxy-6-[(4-nitrophenyl)azo]naphthalene-2,7-disulphonic acid} (I) reacts with vanadium rather than niobium to form a blue complex under suitable conditions.

This paper reports a sensitive and selective spectrophotometric method for the determination of vanadium using sulphochlorophenol N. Attempts to determine a small amount of vanadium in steels have been made.



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Experimental

Apparatus

Absorption spectra were recorded with a Hitachi, Model 124, automatic recording spectrophotometer and other spectrophotometric measurements were made with a Hitachi, Model 139, spectrophotometer, with 1-cm matched cells. A Hitachi-Horiba pH meter, Model F-7ss, was used for the pH measurements.

Reagents

All reagents used were of analytical-reagent grade. De-ionised distilled water was used in all dilutions.

3-[(5-Chloro-2-hydroxy-3-sulphophenyl)azo]-4,5-dihydroxy-6-[(4-nitrophenyl)azo]naphthalene-2,7-disulphonic acid (sulphochlorophenol N). Diazotise 2-amino-4-chlorophenol-6-sulphonic acid (0–5 °C) and couple with an equimolar amount of chromotropic acid in dilute sodium hydroxide solution. Salt-out the monoazo dye with saturated sodium chloride solution, and then couple with an equimolar amount of 4-nitroaniline diazonium salt, in the presence of calcium hydroxide, to produce the bisazo dye.

Allow the reaction mixture to stand at room temperature for 12 h and acidify it by adding concentrated hydrochloric acid. Filter off the precipitate and wash it with hydrochloric acid (1 + 4). Re-precipitate 2–3 times by dissolving in water by the addition of dilute sodium hydroxide solution, followed by hydrochloric acid, until one spot appears on a paper chromatogram, developed with 2 M ammonia solution saturated with butan-2-ol.[11] The extraction method[12] was employed to remove calcium and sodium from the purified compound completely. The yield is approximately 40%.

Dissolve 0.2 g of sulphochlorophenol N in 1 l of de-ionised distilled water. This solution is stable for several months.

Vanadium(V) standard solution. Dissolve 0.5850 g of ammonium metavanadate (NH_4VO_3) in water in a 500-ml calibrated flask to make a 10^{-2} M stock solution. Dilute 100-fold with water to make a working solution. This solution contains 5.1 µg ml^{-1} of vanadium.

Buffer solution, pH 5.0. A 0.1 M acetic acid - 0.1 M sodium acetate solution system was used.

Citric acid solution, 0.1 M. Dissolve 21 g of citric acid in water and dilute to 1 l.

Calibration

Using a pipette, introduce aliquots of the standard vanadium(V) solution containing 0, 10.2, 20.4, 30.6 and 40.8 µg of vanadium into 25-ml calibrated flasks. Add 5 ml of buffer solution (pH 5.0), 1 ml of 0.1 M citric acid solution and 5 ml of 0.02% sulphochlorophenol N solution, then dilute to the mark with distilled water. Allow the mixture to stand for 10 min and measure the absorbance at 627 nm, with a reagent blank solution prepared at the same time as the sample solution as reference. Draw a calibration graph; this should be linear and pass through the origin.

Results and Discussion

Absorption Spectra

Fig. 1 shows the absorption spectra of sulphochlorophenol N and its vanadium(V) complex at apparent pH 5.0. The absorption maximum of the complex occurs at 627 nm, and there

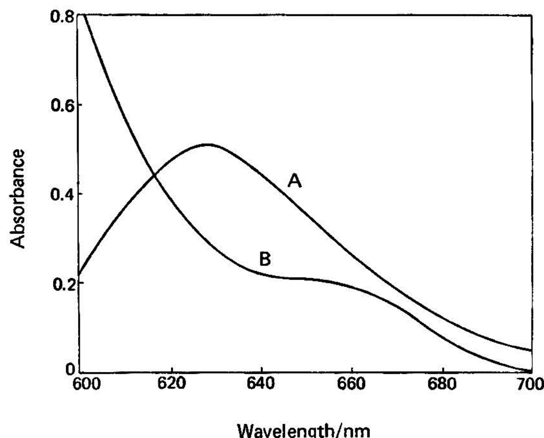

Fig. 1. Absorption spectra: A, vanadium - sulphochlorophenol N complex containing 20.4 µg of vanadium(V) measured against reagent blank; and B, reagent blank measured against water.

was no shift in the wavelength when either the pH was varied from 3.4 to 9.1 or the molar ratio of vanadium to sulphochlorophenol N was varied from 1:5 to 5:1.

Sulphochlorophenol N reacted similarly with vanadium(IV) to form a blue complex (absorption maximum 630 nm) under the same conditions. Further investigation was not carried out because the sensitivity was fairly low compared with the absorption of vanadium(V) complexes.

Influence of pH

The influence of the pH on the absorbance at 627 nm was examined over a pH range from 1.8 to 12.9 (Fig. 2). A maximum and constant absorbance was obtained between pH 3.7 and 6.0. Therefore, the pH was adjusted to about 5.0.

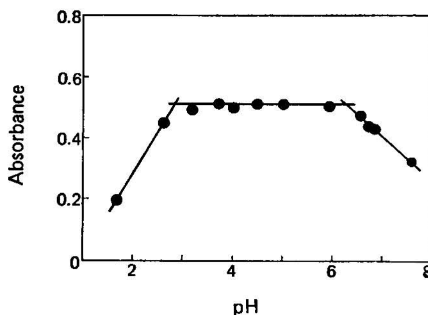

Fig. 2. Effect of pH on absorbance measured at 627 nm.

Reagent Concentration

The effect of the amount of sulphochlorophenol N on the absorbance was examined by varying the molar ratio of sulphochlorophenol N to vanadium(V), the amount of the latter being kept constant (Fig. 3). The results showed that the absorbance of the complex was constant over a 1.5-fold molar excess of the reagent. The recommended volume of reagent, 5 ml, corresponds to a concentration of about 5.7×10^{-5} M in the final solution. This represents a 1.6-fold excess over the highest point on the calibration graph, *i.e.*, 40 μg of vanadium in 25 ml of solution.

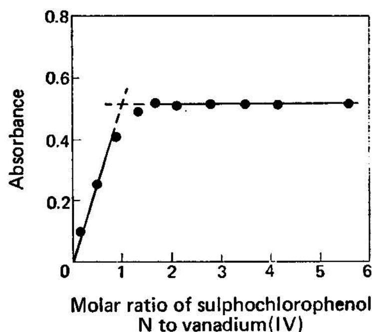

Fig. 3. Effect of reagent concentration on the formation of the vanadium(V) complex.

Effect of Time

The time necessary for complete formation of the sulphochlorophenol N and vanadium complex was examined. Even when a 2-fold molar excess of sulphochlorophenol N was

added to vanadium, about 5 min were sufficient for complete reaction at 20 °C and the absorbance of the complex did not change for at least 2 d. Therefore, the measurement of the absorbance was carried out at least 10 min after addition of the reagent.

Beer's Law

The calibration graph was linear in the range 0–1.6 μg ml^{-1} of vanadium(V), *i.e.*, up to at least 40 μg of vanadium in 25 ml of solution, and its slope corresponded to a molar absorptivity for the complex of 3.12×10^4 l mol^{-1} cm^{-1} at 627 nm.

Composition of the Complex

Attempts to determine the composition of the complex in aqueous solution were made by the continuous-variation and molar-ratio methods (Fig. 3). Both methods revealed that vanadium(V) forms a 1 : 1 complex with sulphochlorophenol N. This is in agreement with Alimarin and Savvin's observation[2] that sulphochlorophenol S forms a 1:1 complex with niobium.

Interferences

The effect of several possible interferences on the determination of 20.4 μg of vanadium is shown in Table I. No interference was noted with large amounts of acetate, bromide, chloride, citrate, fluoride, iodide, nitrate and sulphate. EDTA and tartrate interfered in the determination. The serious interferences arising from cobalt(II) and copper(II) may be caused by complexation with sulphochlorophenol N, which cannot be suppressed by the addition of the usual masking agents.

TABLE I

EFFECT OF DIVERSE IONS ON VANADIUM(V) DETERMINATION

Tests were made by adding the interfering ion to a solution containing 20.4 μg of vanadium(V).

Ion added	Amount of ion added/μg	Amount of vanadium recovered/μg	Recovery, %
Al(III)	20	20.2	99.0
Bi(III)	20	19.9	97.5
Ca(II)	100	20.4	100
Cd(II)	100	20.4	100
Co(II)	20	30.1	148
Cr(III)	1 000	19.6	96.1
Cr(VI)	20	12.6	61.2
Cu(II)	20	33.2	163
Fe(III)	20	21.0	103
Mn(II)	100	19.4	95.1
Mo(VI)	1 000	21.2	104
Nb(V)	100	21.3	104
Ni(II)	20	20.6	101
Pb(II)	100	20.0	98.0
Sb(III)	20	20.4	100
Si(IV)	1 000	21.0	103
Sn(II)	20	20.4	100
Te(IV)	20	20.4	100
Ti(IV)	20	20.2	99.0
Zn(II)	100	20.1	98.5

Application to the Determination of Vanadium in Steel

Two samples of Japanese Standards of Iron and Steel (JSS) were analysed in order to check the validity of the method. When carrying out the determination of vanadium in steels, a large amount of iron(III) also interferes and a suitable preliminary treatment is therefore required. If the vanadium(V) ion is reduced to vanadium(IV), the extraction method of Specker and Doll[13] with 4-methylpentan-2-one should prove satisfactory. Ammonium iron(II) sulphate is suitable for this purpose as a reducing agent.

The preparation of the sample solution is as follows. Transfer a suitable mass (0.05–0.5 g) of the finely divided iron or steel sample into a 100-ml beaker and add 5 ml of water, 5 ml of concentrated hydrochloric acid and 1 ml of concentrated nitric acid. Heat gently until

all of the metal is in solution, then evaporate almost to dryness. Dissolve the residue in 5 ml of concentrated hydrochloric acid and then transfer the solution completely (rinse with 10 ml of water) into a 100-ml separating funnel. Add 10 ml of concentrated hydrochloric acid, about 3 mg of ammonium iron(II) sulphate and 30 ml of 4-methylpentan-2-one, shake the funnel vigorously for a few minutes and allow it to stand for 30 min. Transfer the aqueous phase into an evaporating dish and evaporate to dryness. Add a small amount of concentrated nitric acid and heat again. Dissolve the residue in distilled water and adjust this solution to about pH 5 with 1 N sodium hydroxide solution. Then dilute to 100 ml with distilled water in a calibrated flask. Transfer 1–5 ml of the solution, with a pipette, into a 25-ml calibrated flask and determine the vanadium as described above.

The results obtained are given in Table II. It can be seen that the experimental results are in good agreement with the certified values. Recovery tests were also carried out by adding 10.2 μg of the vanadium standard solution to the steel-sample solution. The mean recovery was 102% with a standard deviation of 0.5%.

TABLE II

DETERMINATION OF VANADIUM(V) IN JSS SAMPLES

Average of at least four determinations.

Sample	Mass of sample/ g	Vanadium content, %		Relative error, %
		Certified value	Found	
JSS 159-3	0.1017	0.31	0.305	−1.6
	0.2001		0.316	+1.9
	0.5003		0.320	+3.2
JSS 852-1	0.0496	0.52	0.528	+1.5
	0.0498		0.534	+2.7
	0.0628		0.495	−4.8

The author is indebted to Professor Kyoji Tôei, Okayama University, for valuable advice and discussions and to Mr. K. Kaichida for technical assistance.

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Received *July 14th*, 1978
Accepted *October 11th*, 1978

Analyst, April, 1979, Vol. 104, pp. 328–333

Oxidative Determination of Dextromoramide (Palfium) in Body Fluids

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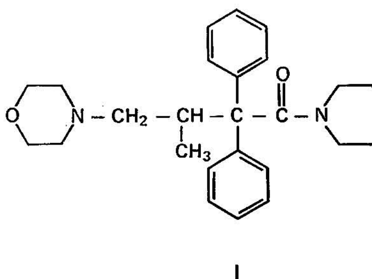
The oxidation of dextromoramide to benzophenone with alkaline potassium permanganate and measurement of its ultraviolet absorbance is advocated for the determination of this drug in urine and serum over the concentration range 5–40 μg ml^{-1}.

Keywords: *Dextromoramide determination; urine; serum; plasma; ultraviolet spectrophotometry*

Although the drug dextromoramide (I) has been used for some years as an analgesic, there is a paucity of information on methods for its assay when present in biological fluids. This gap has caused problems in drug treatment centres[1] and in a forensic[2] context where it has been necessary to identify and determine the drug. Interpretation of any results obtained in these circumstances would also be impossible in view of the lack of information available on the urine and blood levels to be expected after normal therapeutic use. This paper describes an oxidative procedure that may be applied to the determination of this drug and/or metabolites containing the diphenylmethyl moiety, following their extraction from an alkaline solution.



I

Experimental

Reagents

All reagents were of analytical-reagent grade unless otherwise stated.

Instrumentation

All ultraviolet spectra were recorded on a Cecil CE505, double-beam ultraviolet spectrophotometer, using a silica cell with a path length of 2 cm.

Preparation of Calibration Graphs for Dextromoramide in Aqueous Solution

A stock solution containing the equivalent of 500 μg ml^{-1} of dextromoramide base was prepared by dissolving 69.1 mg of dextromoramide tartrate in 100 ml of water. A 20-ml volume of this solution was diluted to 100 ml in order to give a working solution containing 100 μg ml^{-1} of drug base. Aliquots (0.05–0.5 ml) of this working solution were taken and an oxidising solution, prepared by mixing 10 ml of 4% *m/V* potassium permanganate with 15 ml of 9 M sodium hydroxide solution, was added, followed by 5 ml of hexane. The mixture was refluxed for 40 min in a water-bath thermostatically controlled at 70–80 °C.

After cooling in air for 15 min, the condenser was rinsed with water to wash any oxidation product into the flask and the mixture transferred into a glass-stoppered test-tube. An aliquot of the hexane layer was removed and its absorbance over the range 220–280 nm

recorded using hexane, which had been separately refluxed with the oxidising mixture, as reference. This procedure was repeated six times for statistical evaluation.

The above oxidations with alkaline permanganate were repeated using various strengths of sodium hydroxide solution in the range 3–14 M, the last concentration being that described by Caddy *et al.*[3] for the oxidation of methadone.

Preparation of Calibration Graphs for Dextromoramide in Urine

Standard solutions of the drug were prepared as detailed above, using drug-free urine in place of water.

Aliquots (1–10 ml) of the urine solution (containing 5–50 μg of drug base) were pipetted into 100-ml glass-stoppered test-tubes and solid sodium carbonate and sodium hydrogen carbonate (1 + 1 *m*/*m*) added until a saturated solution was obtained. To the alkaline urine an amount of 2,2,4-trimethylpentane - pentan-1-ol (20 + 1 *V*/*V*) was added such that the ratio of the volume of urine sample to that of the extracting solvent was not less than 1:4.

The tubes were shaken for 15 min using a tilt shaker and set aside until the phases separated. The extract was evaporated to dryness under reduced pressure and the residue subjected to the oxidation procedure detailed above. The absorbance of the hexane layer was recorded over the range 220–280 nm using hexane from a similarly treated, drug-free urine sample as reference. This procedure was repeated three times for statistical evaluation.

Preparation of Calibration Graphs for Dextromoramide in Serum

A 2-ml volume of serum was taken in a glass-stoppered test-tube and 0.1–0.8 ml of an aqueous solution containing the equivalent of 100 μg ml^{-1} of dextromoramide base was added followed by 5 ml of 1 M sodium hydroxide solution and 10 ml of 2,2,4-trimethylpentane (reagent grade). The mixture was shaken for 15 min using a tilt shaker. Any emulsion formed was completely cleared by adding 5 ml of 2,2,4-trimethylpentane - pentan-1-ol (20 + 1 *V*/*V*). The organic layer was separated and evaporated to dryness under reduced pressure and the residue oxidised with alkaline permanganate as detailed for dextromoramide in aqueous solutions. Following oxidation, the absorbance of the hexane layer was recorded over the range 220–280 nm using a hexane extract from drug-free serum that had been similarly treated, as the reference solution. The procedure was repeated nine times for statistical evaluation.

Oxidation of Dextromoramide with Other Oxidising Agents

Solutions of dextromoramide base containing 20 μg of the drug were oxidised with aqueous acidic dichromate solution,[4] non-aqueous acidic (glacial acetic acid - concentrated sulphuric acid) chromium(VI) oxide solution[5] and acidic cerium(IV) sulphate solution,[6] the last procedure being modified by replacing hydrochloric acid with sulphuric acid. Dextromoramide (20 μg) was oxidised with barium peroxide in 66% *m*/*V* sulphuric acid and 9 M sodium hydroxide solution as described by Wallace *et al.*[7]

Drug Regime and Biological Sampling

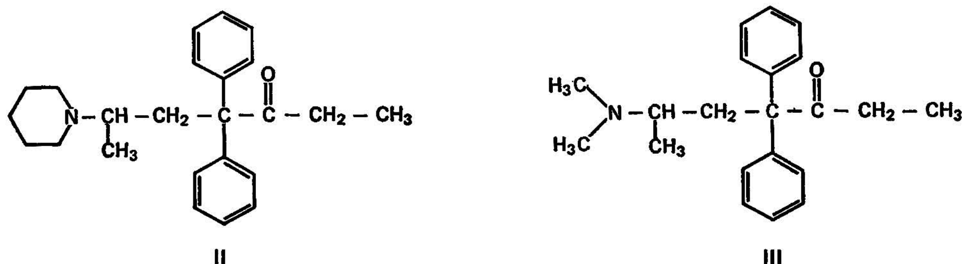
A single oral therapeutic dose of Palfium (dextromoramide tartrate, 5 mg) was administered to a patient. Serum (10 ml) and urine (25 ml) samples were obtained at hourly intervals over a period of 24 h and one sample at 48 h. These samples were analysed by the alkaline permanganate oxidation procedure detailed above.

Results and Discussion

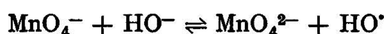
Oxidation of Dextromoramide with Alkaline Permanganate

Caddy *et al.*[3] reported that a concentration of 14 M sodium hydroxide solution is the optimum for the oxidation of dipipanone and methadone with alkaline permanganate.

As dextromoramide (I) is structurally similar to both dipipanone (II) and methadone (III), it might be expected that the same conditions would be applicable to its oxidation to benzophenone with alkaline permanganate. This was found to be so, but reproducible quantitative results were not given by this procedure. However, a problem associated with



the use of sodium hydroxide solution at this concentration is that the decomposition of alkaline permanganate to manganate occurs with great facility. This can be explained in terms of the reversible reaction



$$MnO_4^- + HO^- \rightleftharpoons MnO_4^{2-} + HO^{\bullet}$$

proposed by Symons[8,9] and by Jezowska-Trezebiatowska *et al.*[10] as the initiating reaction in a multi-step mechanism proposed for the decomposition of alkaline permanganate to manganate and oxygen. An increase in concentration of sodium hydroxide will therefore facilitate the decomposition of permanganate. When using 14 M sodium hydroxide solution, it was observed that the oxidising mixture turned green (indicating the presence of the manganate species) before the oxidation of dextromoramide was completed.

The use of 9 M sodium hydroxide solution and an increase in the ratio of permanganate to alkali solution from 1 + 3 (*V*/*V*) to 2 + 3 (*V*/*V*) was found to be optimum for the oxidation of dextromoramide (Fig. 1). This concentration of reagents also ensured that there was an excess of permanganate in the mixture at the end of the oxidation procedure. Further, it was observed that the hexane layer from mixtures that were green owing to the presence of manganate had an unusually high absorbance at 247 nm compared with hexane from mixtures that still had an excess of permanganate after the oxidation.

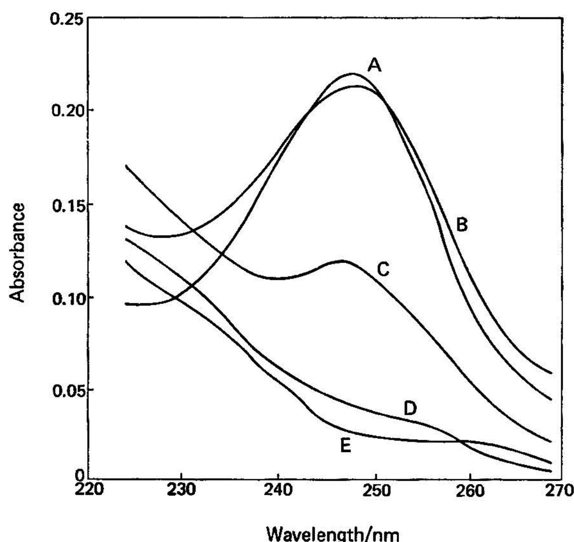


Fig. 1. Typical spectra of the oxidation product in hexane obtained from 30 µg of dextromoramide originally present in: A, aqueous solution; B, urine; and C, serum; together with D, serum blank; and E, urine blank.

The probable explanation for this observation is that the permanganate was reduced by the organic drug molecule to give the hypomanganate (MnO_4^{3-}), which in the strongly alkaline medium disproportionated to manganate and manganese dioxide:

$$2MnO_4^{3-} + 2H_2O \rightleftharpoons MnO_4^{2-} + MnO_2 + 4HO^-$$

A fine suspension of manganese dioxide in the hexane layer scattered the light during the ultraviolet measurements, thereby giving rise to the apparent high values of absorbance observed.

In the presence of excess of permanganate, any hypomanganate formed was instantaneously oxidised and the formation of manganese dioxide prevented.

Preparation of Calibration Graphs for Dextromoramide in Aqueous Solution and in Urine

The choice of extraction solvent is dictated by the need to avoid those solvents (*e.g.*, chloroform) which, even in trace amounts, might interfere by reacting with the oxidant, thereby lowering the yield of benzophenone from the drug. The most effective solvent for this purpose was found to be heptane or 2,2,4-trimethylpentane containing a small amount of pentan-1-ol, 2,2,4-trimethylpentane being preferred for urine as heptane gave high blank values. At the solvent evaporation stage it is also most important to remove the last traces of pentan-1-ol.

The use of a solid mixture of sodium carbonate and sodium hydrogen carbonate to adjust the pH of the urine was found to be advantageous in avoiding emulsification during the extraction procedure. Typical ultraviolet spectra of the oxidation product, which also show appropriate blanks, read against hexane are given in Fig. 1.

Statistical evaluation of the graphs obtained from both aqueous (Table I) and urinary (Table II) samples by comparison of their variances (F = 0.004) shows there is no significant difference between the two graphs and therefore the extraction of the drug from urine is complete. This finding is consistent with simple weighing experiments using 50-mg samples, which show an extracting efficiency of 97%. A value of 114% was obtained from the ratio of the slopes of the two regression lines but it is not possible to calculate the standard deviation of this value.

Calibration graphs of absorbance of the hexane layer at 247 nm against concentration of the drug in micrograms per millilitre in water have good linearity and reproducibility over the range 5–50 μg ml^{-1} and can be used in the determination of the drug in urine.

Preparation of Calibration Graphs for Dextromoramide in Serum

The choice of extraction solvent was determined by the same considerations as stated for

TABLE I

RELATIONSHIP BETWEEN THE CONCENTRATION OF AQUEOUS SOLUTIONS OF DEXTROMORAMIDE AND THE ABSORBANCE OF ITS ALKALINE PERMANGANATE OXIDATION PRODUCT IN HEXANE

Amount of dextromoramide base/μg*	Absorbance of hexane layer at 247 nm†	Standard deviation
5	0.058	0.013
10	0.087	0.013
20	0.176	0.037
30	0.230	0.032
40	0.287	0.026
50	0.373	0.014

Correlation coefficient = 0.975 ± 0.015 (95% confidence limits).
Regression equation: $y = 0.024 + 0.007x$, where y = absorbance of hexane layer at 247 nm and x (μg) = amount of dextromoramide base oxidised.
Standard error of y = 0.004.

* Actual amount of drug oxidised.
† All values are the means of six determinations.

TABLE II

RELATIONSHIP BETWEEN THE CONCENTRATION OF DEXTROMORAMIDE IN URINE AND THE ABSORBANCE OF ITS OXIDATION PRODUCT IN HEXANE

Amount of dextromoramide base/μg*	Absorbance of hexane layer at 247 nm†	Standard deviation
5	0.033	0.032
10	0.094	0.034
20	0.173	0.003
30	0.264	0.048
40	0.315	0.019
50	0.414	0.010

Correlation coefficient = 0.965 ± 0.025 (95% confidence limits).
Regression equation: $y = 0.002 + 0.008x$; x and y as stated in Table I.
Standard error of $y = 0.009$.

* Actual amount of drug oxidised.
† All values are the means of three determinations.

the preparation of the calibration graph for dextromoramide in urine. The 2,2,4-trimethylpentane - pentan-1-ol mixture used for the extraction of urine was unsuitable for use in the extraction of serum as it gave rise to high blank values in the final hexane solution and recourse was made to 2,2,4-trimethylpentane alone. The recovery of dextromoramide from serum using this solvent was found to be 36% as measured from the ratio of the slopes of the regression lines. This value is lower than would be expected from simple weighing experiments using 50-mg samples of drug extracted from plasma (68%) and may be either a reflection of the efficiency of oxidation for this type of sample or more simply because of the reduction in solvent polarity that results from the omission of the pentan-1-ol. However, the extract was clean and results were reproducible. The analytical results are given in Table III.

A typical ultraviolet spectrum of the oxidation product produced from the oxidation of an extract of serum, together with an appropriate blank, read against hexane, is shown in Fig. 1.

TABLE III

RELATIONSHIP BETWEEN THE CONCENTRATION OF DEXTROMORAMIDE IN SERUM AND THE ABSORBANCE OF ITS OXIDATION PRODUCT IN HEXANE

Concentration of dextromoramide base*/ μg ml^{-1}	Absorbance of hexane layer at 247 nm†	Standard deviation
5	0.022	0.0062
10	0.049	0.0103
20	0.094	0.0192
30	0.146	0.0410
40	0.208	0.025

Correlation coefficient = 0.94 ± 0.03 (95% confidence limits).
Regression equation: $y = 0.0051 + 0.0026x$; x and y as in Table I.
Standard error of $y = 0.0035$.

* Original concentration of drug in a 2-ml sample of serum.
† All values are means of nine determinations.

Serum and Plasma Levels Found in a Patient Administered a Single Therapeutic Dose of Palfium

The methods described above have been applied successfully to the determination of dextromoramide and/or its metabolites containing the diphenylmethyl moiety present in the urine and serum of a patient administered a single oral therapeutic dose of the drug. The levels found are given in Table IV. The time of maximum serum concentration is 4 h and that for urine 5 h. Interestingly the concentration in the serum is still higher than

TABLE IV

URINE AND SERUM LEVELS OF DEXTROMORAMIDE FOUND OVER A 48-h PERIOD FOLLOWING THE INGESTION OF A SINGLE THERAPEUTIC DOSE

The results also include any metabolites containing the diphenylmethyl group.

Time/h	1	2	3	4	5	6	7	8	24	48
Serum concentration/μg in 10 ml	21	23	28.5	29.5	29	24.5	22.8	21	18.5	18
Urine concentration/μg in 10 ml	13.5	14	14.5	14.9	20	8.5	4.1	5	6.5	8

that in the urine even after 48 h. It is hoped to present more excretion data in a further paper.

Oxidation of Dextromoramide with Other Oxidising Agents

The use of reagents other than alkaline permanganate for the oxidation of drugs containing either the diphenylmethyl grouping or an aromatic ring with a suitable side-chain have been reported by several workers.[4–7] As many of these compounds, especially in a forensic context, may be present in body fluids together with dextromoramide, it is necessary to establish if this latter drug is oxidised to benzophenone under the conditions used for the oxidative assay of these other drugs. Other reagents, such as acidified dichromate,[4] chromium(VI) oxide[5] and acidified cerium(IV) sulphate,[6] used for the oxidation of diphenhydramine[4,5] and dexamphetamine,[6] respectively, and barium peroxide,[7] used for the oxidative assay of methadone, failed to oxidise dextromoramide. Although this failure to oxidise dextromoramide (**I**) to benzophenone with acidic oxidising agents can be explained by the absence of the requisite structural features, it is difficult to account for the lack of formation of benzophenone when dextromoramide is oxidised with barium peroxide, especially in view of its structural similarity to methadone (**III**). As barium peroxide has rarely been employed as an oxidant in organic chemistry, the answer may lie in the mode of action of this reagent, which is at present unknown.

Use can be made of the selectivity of barium peroxide as oxidant for methadone in an assay for this drug in admixture with dextromoramide. Using the conditions detailed above, a methadone - dextromoramide mixture was first oxidised with barium peroxide and then with alkaline permanganate. The difference between the absorbances of the benzophenone in the hexane solution was found to be a measure of the amount of dextromoramide present.

Conclusion

Oxidation of dextromoramide with alkaline permanganate and subsequent measurement of the absorbance of the benzophenone obtained provides a satisfactory method for the assay of the drug and/or its metabolites containing the diphenylmethyl moiety in biological fluids. Satisfactory calibration graphs were obtained for the drug over the concentration ranges reported.

Other oxidation methods investigated were of no analytical value in the oxidative determination of dextromoramide, as the different oxidising agents failed to convert dextromoramide to benzophenone.

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Received *April 24th*, 1978
Accepted *October 19th*, 1978

Reproducibility of Pyrolysis - Mass Spectrometry Using Three Different Pyrolysis Systems

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A study has been made into some of the factors that affect the reproducibility of pyrolysis - mass spectrometry. Three separate pyrolysis systems were examined and three sample types, a simple system of easily pyrolysable polymers, an acrylic paint and an alkyd paint, were employed in order to cover a range of ease of sample pyrolysis. These samples were also examined by pyrolysis - gas chromatography.

The reproducibility of the pyrolysis - mass spectrometry system was found to vary according to sample type. The source of irreproducibility was identified as the pyrolysis process and not the mass spectrometric detection.

Keywords: *Reproducibility*; *pyrolysis - mass spectrometry*; *pyrolysis - gas chromatography*; *paint analysis*

Although the combination of pyrolysis with mass spectrometry was investigated by Zemany[1] in 1952, and by other workers[2,3] at the same time, it is only in recent years that pyrolysis - mass spectrometry (Py - MS) has become established as a technique for examining polymeric material. The pioneering work of Meuzelaar and co-workers concentrated on the field of complex bio-organic substrates, notably bacteria,[4,5] but also humic acids[6] and peptides.[7] Bacterial identification by Py - MS has become an accepted procedure,[8–10] and the technique has been used in examining other naturally occurring organic materials, such as polysaccharides.[11] The early work on synthetic polymers,[2] including polystyrene,[3] has been extended to include aromatic and aliphatic polyesters[12] and vinyl polymers and copolymers.[13] Zeman[14] has reported the pyrolysis - mass spectrometry of 25 commercially available polymers.

The attraction of Py - MS as a technique providing useful data on small samples of polymeric material has led to its application in forensic science.[15–18] In this context the technique has been applied successfully to the identification and comparison of paint and fibre samples.

In Py - MS a sample is broken down pyrolytically (either within or external to the ionisation source of the mass spectrometer) and then repetitive mass spectral scans of the pyrolysate are taken. Data processing facilities enable a number of scans to be accumulated, possibly followed by integration to produce a mass pyrogram of the sample.[15]

If Py - MS is to be used on a routine basis, involving perhaps the comparison of a mass pyrogram of a sample with a library collection of mass pyrograms, then the reproducibility of the system becomes an important factor. Good analytical reproducibility will be essential for any computer-based method of comparing mass pyrograms, and it is especially important when electron-impact (EI) mass spectrometry is used. In this mode the fragmentation within the source of the mass spectrometer gives many small fragment ions and leads to complex mass pyrograms. Simpler mass pyrograms would result from the use of chemical-ionisation (CI), field-ionisation (FI) or low-voltage EI mass spectrometric detection, as these methods give molecular ions. Although, at the qualitative level, it would be easier to detect differences between samples by visual inspection of CI mass pyrograms compared with the corresponding EI mass pyrograms, EI mass spectrometry has the advantages of better sensitivity, providing more information and ease of controlling the experimental conditions, particularly with respect to gas pressure within the ionisation source.

The two processes occurring in a Py - MS analysis, the pyrolysis and the mass spectrometric fragmentation, can give rise to irreproducibility, but few of the papers published in this field have investigated the reproducibility of the procedure. Reiner is quoted[9] as saying that the reproducibility of mass spectrometry is not as good as that obtained with gas chromatography (both in conjunction with pyrolysis) owing to the tendency of the mass spectrometer ionisation source to become contaminated. Meuzelaar *et al.*,[19] in characterising

bacteria, reported that the variations caused by culturing the bacteria, sampling and analysing by Py - MS were less than the differences between strains. The reproducibility was not measured, although the authors found that results taken prior to a complete overhaul of the mass spectrometer were similar to those obtained afterwards. Acceptable reproducibility was also claimed by Zeman[14] when he subjected synthetic polymer samples to programmed heating on a direct probe in a mass spectrometer operating under EI conditions. He found that the polymers tested could be degraded reproducibly in the temperature range 160–500 °C.

Quantitative measures of the reproducibility of Py - MS systems have been quoted by Schulten and Görtz[11] and by Saferstein and Manura.[17] Schulten and Görtz used Curie-point pyrolysis in combination with high-resolution FI mass spectrometry and found the variation for repetitive pyrolyses of glycogen samples to be about $\pm 5\%$ for peaks of intensity greater than 10%. Saferstein and Manura,[17] pyrolysing under CI conditions, found that their mass pyrograms were reproducible to $\pm 10\%$ at each mass unit, but this reproducibility could only be maintained over a 6-h period.

Irwin and Slack[20] have noted the lack of reproducibility data for Py - MS and as irreproducibility in this type of analysis has been quoted[16,18] as a limiting factor in its routine use in forensic work, the purpose of the present work was to assess the reproducibility of the technique. Three sample types were pyrolysed on three separate pyrolysis systems, coupled in turn to one mass spectrometer; concomitant analyses of the sample types were effected by pyrolysis - gas chromatography (Py - GC).

Experimental

Equipment

(a) Pyrolysis - gas chromatography

Instrument	Varian Aerograph, Series 1400
Column	12 ft × 1/8 in o.d. stainless steel packed with 15% Carbowax 20M on Gas-Chrom Q (80–100 mesh)
Carrier gas	Nitrogen, flow-rate 25 ml min^{-1}
Detector	Flame ionisation
Temperatures	Detector 220 °C; injector 200 °C; oven 190 °C isothermal for pyrolysis of wires coated with polyvinyltoluene - polystyrene solution; temperature programmed from 70 to 190 °C at 10 °C min^{-1} for paint samples
Pyrolyser	Pye Curie-point, 15-s pyrolysis at 610 °C

(b) Mass spectrometry

Instrument	VG Micromass 12F (a magnetic sector mass spectrometer)
Electron impact conditions:	
electron energy	70 eV
emission current	100 μA
accelerating voltage	4 kV
source temperature	230 °C
mass range	approximately 35–250 a.m.u.
scan rate	1 s per decade with magnet re-set time of 1 s
Data acquisition	VG 2040 data system
Pyrogram read-out	Bryans 26000 A4 *X* – *Y* plotter

(c) Pyrolysis systems for mass spectrometry

(*i*) Pye Curie-point pyrolyser, 15-s pyrolysis at 610 °C.
(*ii*) Chemical Data Systems (CDS) Pyroprobe 100, 20-s pyrolysis at 700 °C (nominal temperature).

The instrumental system employed for these two pyrolysers was influenced by the need to switch rapidly to normal gas chromatograph - mass spectrometer operation, and it is essentially the system of Hughes *et al.*[15] The two pyrolysers could be connected alternately

to an empty 45 cm × 6.35 mm o.d., 2 mm i.d. glass column, maintained at 200 °C (in the GC oven), and were operated with a purging helium flow-rate of 5 ml min^{-1} (mass flow). A 5 ml min^{-1} make-up flow of helium was introduced at the end of the glass column connected to a length of stainless-steel tubing, 0.159 mm o.d. A restrictor (of stainless-steel tubing) was inserted between this stainless-steel tubing and the length of glass-lined stainless-steel microbore tubing that led to the jet separator of the mass spectrometer. The restrictor could be adjusted (*e.g.*, after cleaning the jets) to ensure that the pyrolysate arrived in the mass spectrometer at a reproducible time after pyrolysis.

(*iii*) VG pyrolysis probe, 15-s pyrolysis at 610 °C.

This probe,[21] which fitted in place of the direct insertion probe, incorporated a Curie-point pyrolyser. The main difference between this system and systems (*i*) and (*ii*) was that the pyrolysis products passed directly into the ionisation source of the mass spectrometer. Pyrolyses with this system were carried out with no flow in the line connected to pyrolysis systems (*i*) or (*ii*) and with the dump valve closed.

Samples

Three sample types were chosen to cover a range of ease of sample pyrolysis.

(*a*) A simple system of easily pyrolysable polymers. Polymer-coated wires were prepared by dipping Pye 610 °C Curie-point wires into a solution of polyvinyltoluene and polystyrene in tetrahydrofuran to a depth of approximately 1 cm. The sample loading was estimated as being about 200 ng.

(*b*) An acrylic paint sample, known from Py - GC studies to pyrolyse reproducibly (International Pinchin Johnson, Canary Yellow).

(*c*) An alkyd paint sample, known from Py - GC studies to pyrolyse in a variable manner (Permoglaze, White Gloss).

For sample types (*b*) and (*c*) the masses of the fragments of dried paint used for the Py - GC studies were in the range 10–30 µg, while for the Py - MS studies the masses were in the range 3–12 µg.

Method

The reproducibility experiments were carried out over a 6-week period; during this time the mass spectrometer was also being used in its GC - MS mode for the routine identification of drugs in body fluids. On each day of the reproducibility study duplicate samples of the two paint types were run on each of the four pyrolysis systems, and five polymer-coated wires were run on the three Curie-point systems.

Several instrumental variables were monitored each day in case an instrumental malfunction produced irreproducible results. Readings of the source and backing pressures were taken, and the levels of the nitrogen and oxygen peaks were monitored, in order to detect (and thence remove if necessary) any appreciable air leaks in the system.

Each day, before use with the pyrolyser, the mass spectrometer was tuned on a sample of toluene (0.4 µl injected through the vapour-phase inlet) to give the spectrum m/e (%I): 91 (100); 92 (71–74); 65 (11–13); 63 (5–6); 51 (5–6); and 93 (5).

The procedure adopted in the Py - MS experiments was to introduce the sample into the pyrolyser and allow the system to stabilise for approximately 3 min before initiating the repetitive mass spectral scanning. Background spectra were then collected for 20 s before pyrolysing. Between 30 and 60 spectra were collected, depending on the pyrolysis system and sample type. Using the on-line computer facility, background subtraction was followed by data manipulation using the specially written FORTRAN IV programs.[15] The intensities of the ions at m/e 28, 32, 40 and 44 were set to zero, and several scans were averaged to produce mass pyrograms. Twelve mass spectral scans were averaged for pyrolysis systems (*i*) and (*ii*), while 32 scans were averaged when the VG pyrolysis probe was used; this was increased to 52 scans for the pyrolysis of the alkyd paint samples, in order to increase the proportion of phthalic anhydride and benzoic acid detected.

As some mass spectrometer facilities may not have access to computers for data reduction, single scans were examined at a fixed time interval after pyrolysis (in fact a scan shortly after the maximum total ion current had occurred). These scans would give a measure of the reproducibility attainable without use of a data system.

Results

Mass Spectrometer Tuning

Over the period of the experiment two sources were used. One source could be tuned easily to give a consistent standard toluene spectrum. This source has subsequently been removed from and returned to the mass spectrometer several times, for dismantling and cleaning, but it is still capable of being tuned to the standard toluene spectrum.

The second source gave a lower sensitivity (by a factor of 3) at its optimum tuning position, but at this position it gave a different toluene spectrum. The standard toluene spectrum could be obtained by tuning this source, but a further (3-fold) decrease in sensitivity occurred, and the spectrum suffered from erratic intensity ratios. As the pyrolysis results obtained with the second source were distinctly different from those obtained with the first source, only the results from the first source are considered in this reproducibility study. It has yet to be determined whether the source discrepancy is basic to the two sources, or whether the second source contained a fault.

Pyrolysis of Polymer-coated Wires

Under the experimental conditions the wires coated with polyvinyltoluene - polystyrene solution pyrolysed to give essentially the vinyltoluene and styrene monomers (see Fig. 1 for the pyrolysis - gas chromatogram). The pyrolysate mass spectrum, *m/e* (%*I*): 104 (100), 118 (66), 117 (63), 103 (50) and 78 (34), included ions at *m/e* 104 and 103 arising from styrene and ions at *m/e* 118 and 117 from vinyltoluene [in each instance the ions represented the molecular ion and (molecular ion —1)]. Monitoring the ratios of ions 118/104 and 117/118 gave a measure of the inter- and intra-compound variation, respectively. The results of both the Py - GC and Py - MS experiments with this sample type are summarised in Table I.

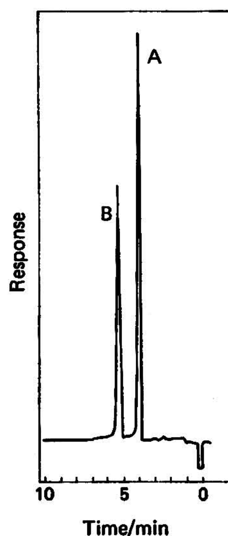

Fig. 1. Pyrolysis - gas chromatogram of polymer-coated pyrolysis wire. A, Styrene; and B, vinyltoluene.

The results show the following:

(*i*), averaging a pyrolysis run over 12 scans improves the reproducibility of the two Py - MS systems by a factor of two;

(*ii*), the over-all reproducibility of ion measurement (after pyrolysis plus mass spectrometric processes) for ions from a single compound is better than that for ions from different compounds;

TABLE I

CURIE-POINT PYROLYSIS FOR POLYMER-COATED WIRES

Ion	Ratio, % Pyrolysis into glass column (30 samples)	VG pyrolysis probe (30 samples)	Pyrolysis - gas chromatography (32 samples)
Mass spectrometer runs averaged (12 *scans*), *relative standard deviations given in parentheses*—			
118/104	67.3 (2.5%)	62.7 (5.5%)	56.7 (3.1%)
117/118	92.8 (1.8%)	90.8 (3.2%)	
Single scan considered, relative standard deviations given in parentheses—			
118/104	75.6 (5.8%)	62.4 (9.9%)	
117/118	95.3 (6.2%)	92.4 (8.4%)	
	(Scan No. 18)	(Scan No. 15)	

(*iii*), the reproducibility of measurement of the vinyltoluene - styrene ratio on Py - GC is approximately the same as that for Py - MS using the glass column and is approximately twice as good as Py - MS with the VG pyrolysis probe. The intra-compound reproducibility is also worse for the VG pyrolysis probe. This irreproducibility probably arises from the small sample sizes, and the longer time period of introduction of the pyrolysate into the mass spectrometer, for the pyrolysis probe (Fig. 2).

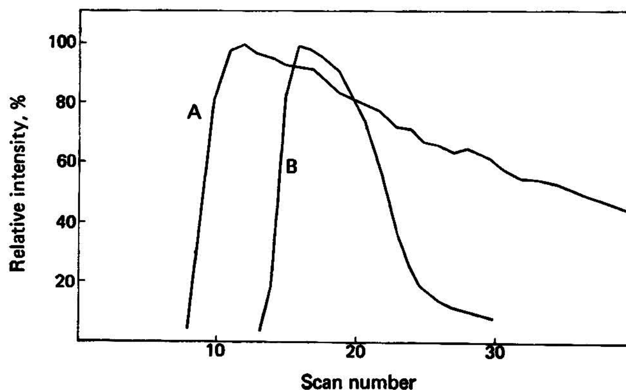

Fig. 2. Total ion current profiles for two pyrolysis systems using polymer-coated wires. A, VG pyrolysis probe; and B, Curie-point pyrolyser into glass column.

Pyrolysis of Acrylic Paint Samples

Py - GC (see Fig. 3) indicated that pyrolysis of this sample gave three major components, *viz.*, butan-1-ol, butyl methacrylate and styrene, and the Py - MS results are consistent with this observation. The results for the Py - GC experiments are given in Table II and in Tables III–V the Py - MS results are summarised. For the Py - MS study, ten ions were monitored with their intensities normalised to m/e 41 as 100%: m/e 39, 41, 55, 56, 57, 69, 78, 87, 103 and 104. Some of these ions could arise from more than one compound, but the ions at m/e 103 and 104 were characteristic of styrene; the ratio of 103 to 104 was used to examine the intra-compound variation. Ions from this group appearing in the spectra of the major components are: [m/e (% I)] butan-1-ol 56 (100), 41 (54), 55 (14); butyl methacrylate 41 (100), 39 (59), 69 (58), 56 (33), 87 (32), 57 (11); and styrene 104 (100), 103 (38), 78 (31), 39 (11).

As a means of measuring the similarity of pairs of (averaged) mass pyrograms, FIT factors were calculated. The definition of the FIT factor[15]:

$$\text{FIT} = 1000\left[1 - \frac{\Sigma(I_x - I_y)^2}{\Sigma(I_x^2 + I_y^2)}\right]$$

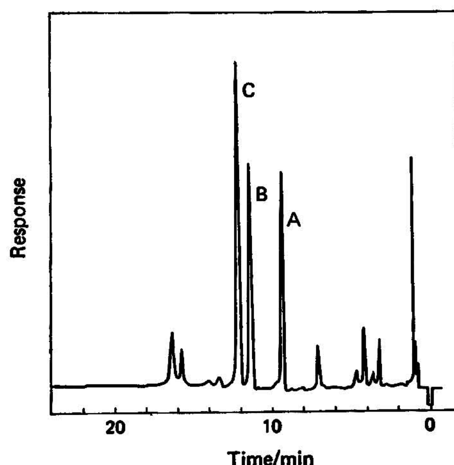

Fig. 3. Pyrolysis - gas chromatogram of acrylic paint sample. A, Butan-1-ol; B, butyl methacrylate; and C, styrene.

TABLE II

PYROLYSIS - GAS CHROMATOGRAPHY OF ACRYLIC PAINT

Proportion of major components (relative to styrene), relative standard deviations given in parentheses.

Variation	Butan-1-ol	Butyl methacrylate	Styrene
In-day variation (11 samples) ..	72.6 (1.8%)	66.2 (2.6%)	100
Long-term variation (14 samples) ..	71.5 (2.3%)	69.3 (1.9%)	100

TABLE III

Py - MS OF ACRYLIC PAINT USING CURIE-POINT PYROLYSIS INTO GLASS TUBE

In-day variation: 10 samples, 12 spectra averaged for each run.

Ion (*m/e*)	39	41	55	56	57	69	78	87	103	104	103/104
Intensity	31.9	100	33.8	66.4	36.2	61.8	22.1	35.0	28.5	66.0	43.2
Relative standard deviation, %	1.5	—	1.9	2.9	2.5	1.2	2.0	1.4	1.9	1.7	1.3

FIT factors (45 comparisons) average 999.8, worst 999.

Long-term variation: 13 samples, 12 spectra averaged for each run.

Ion (*m/e*)	39	41	55	56	57	69	78	87	103	104	103/104
Intensity	29.9	100	33.7	74.1	36.4	58.2	20.8	34.0	28.7	66.1	43.4
Relative standard deviation, %	7.2	—	6.0	4.6	4.9	4.6	4.1	7.2	9.9	9.6	1.7

FIT factors (36 comparisons) average 996, worst 991.
Four samples were omitted from the FIT factor calculations as they suffered interference from the *m/e* 43 ion during background subtraction.

Long-term variation: 13 samples, single scan.

Ion (*m/e*)	39	41	55	56	57	69	78	87	103	104	103/104
Intensity	31.3	100	37.8	62.5	43.8	66.5	25.1	40.1	35.6	81.7	42.5
Relative standard deviation, %	9.7	—	12	6.7	10	6.6	13	7.6	17	16	3.1

TABLE IV

Py - MS OF ACRYLIC PAINT USING CDS PYROPROBE PYROLYSIS INTO GLASS TUBE

In-day variation: 10 samples, 12 spectra averaged for each run.

Ion (*m/e*)	39	41	55	56	57	69	78	87	103	104	103/104
Intensity	39.5	100	44.4	51.6	39.1	52.6	28.4	27.2	37.4	82.1	45.6
Relative standard deviation, %	1.9	—	1.8	2.5	4.2	5.7	2.9	5.9	3.3	2.6	1.2

FIT factors (45 comparisons) average 998, worst 993.

Long-term variation: 14 samples, 12 spectra averaged for each run.

Ion (*m/e*)	39	41	55	56	57	69	78	87	103	104	103/104
Intensity	35.8	100	41.9	54.7	38.3	54.5	25.9	28.0	36.6	81.3	45.1
Relative standard deviation, %	5.2	—	4.4	3.9	4.6	6.0	4.3	3.8	6.8	7.3	1.4

FIT factors (91 comparisons) average 996, worst 987.

Long-term variation: 14 samples, single scan.

Ion (*m/e*)	39	41	55	56	57	69	78	87	103	104	103/104
Intensity	37.4	100	44.3	54.8	39.0	50.9	29.7	25.1	42.6	93.2	45.8
Relative standard deviation, %	5.9	—	3.8	5.8	6.1	10	3.4	12	4.7	4.7	3.0

where I_x and I_y are the intensities of a given ion (normalised to the total ion current of a particular pyrogram) in the two sets of data, means that perfectly matched pyrograms would give an FIT factor of 1000.

From Table V it can be seen that there are no results given for the in-day variation with the VG pyrolysis probe. The in-day experiment was carried out after the long-term experiments, and after the probe had been used for a considerable amount of pyrolysis - CI work. The variation obtained was extreme, and was attributed to a layer of carbon having been deposited on the inside of the probe line. If pyrolysis - mass spectrometry was to be carried out routinely with the VG pyrolysis probe, then regular cleaning of the line would probably be necessary for optimum performance.

The results given in Tables II–V show: the paint sample pyrolyses reproducibly for the three major pyrolysis products, as measured by Py - GC; the *m/e* 103/104 ratio agrees between the three mass spectrometer pyrolysis systems over a long period of time, indicating that the variation originating in the mass spectrometer should affect the over-all reproducibility by no more than 1–2%; and good reproducibility was achieved with the two systems pyrolysing into the glass column for both the short-term and the long-term experiments. The VG pyrolysis probe gave poorer results than the other two Py - MS systems, and this could be caused by some variation in the line temperature of the probe. In addition, the

TABLE V

Py - MS OF ACRYLIC PAINT USING THE VG PYROLYSIS PROBE

Long-term variation: 11 samples, 32 spectra averaged for each run.

Ion (*m/e*)	39	41	55	56	57	69	78	87	103	104	103/104
Intensity	37.9	100	36.9	50.5	30.2	50.9	27.1	30.0	34.5	76.3	44.4
Relative standard deviation, %	4.4	—	4.4	6.8	6.8	6.3	6.7	7.0	10	9.7	2.1

FIT factors (55 comparisons) average 995, worst 982.

Long-term variation: 13 samples, 32 spectra averaged for each run.

	39	41	55	56	57	69	78	87	103	104	103/104
Relative standard deviation, %	5.3	—	5.0	9.6	10	9.1	9.5	10	13	13	2.9

FIT factors (78 comparisons) average 991, worst 966.

Long-term variation: 13 samples, single scan.

Ion (*m/e*)	39	41	55	56	57	69	78	87	103	104	103/104
Intensity	38.0	100	35.2	48.2	28.3	48.7	27.2	28.9	35.4	79.5	44.4
Relative standard deviation, %	6.3	—	9.5	13	15	12	10	15	16	13	5.1

spread in the time the pyrolysate takes to reach the mass spectrometer (Fig. 2) means that small samples achieve only a small total ion current and give more variable ion ratios. The influence of this factor is shown in the long-term variation experiments with the VG pyrolysis probe, where an improvement in reproducibility was observed after the omission of the data from two small samples that gave low total ion currents.

Typical mass pyrograms for the acrylic paint samples on the three Py - MS systems are given in Fig. 4. FIT factors between these three mass pyrograms are less than 900.

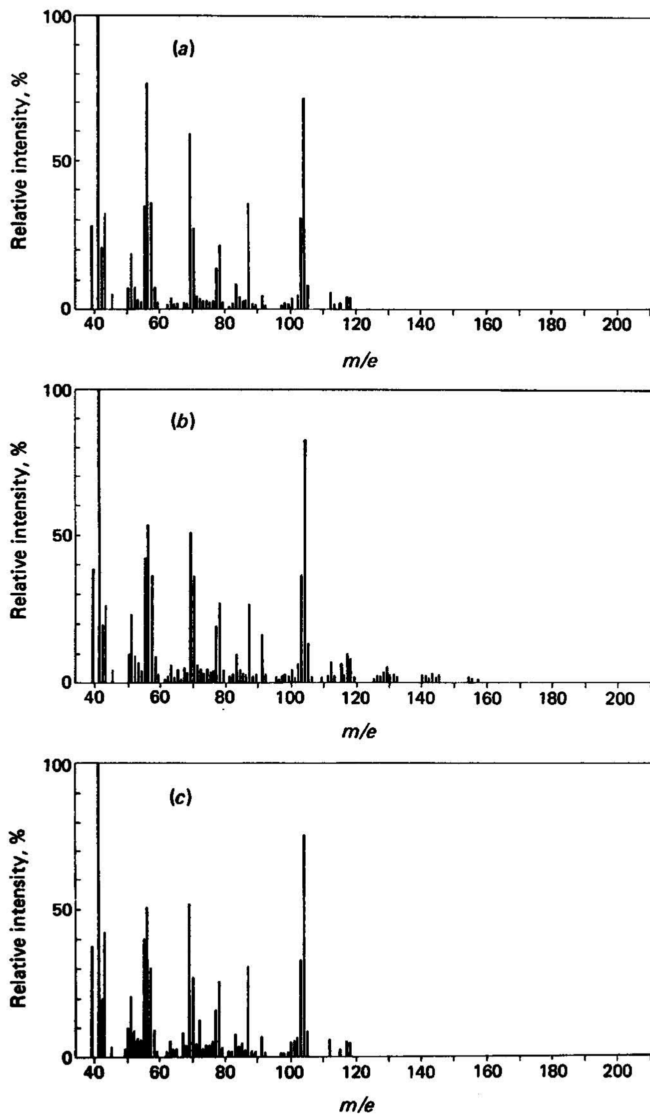

Fig. 4. Mass pyrograms for yellow acrylic paint. (*a*), Curie-point pyrolysis into glass column; (*b*), CDS Pyroprobe into glass column; and (*c*), VG pyrolysis probe.

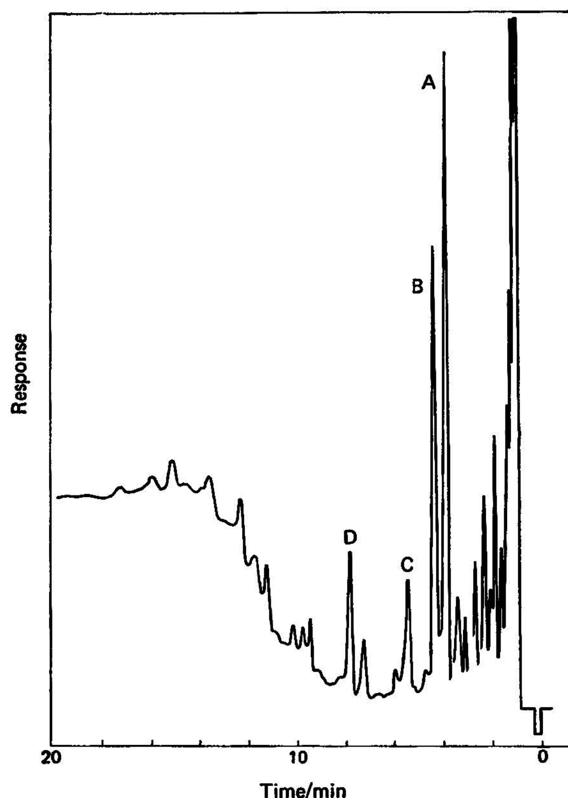

Fig. 5. Pyrolysis - gas chromatogram of alkyd paint sample. A, Acrolein; B, methacrolein; C, benzene; and D, unidentified component.

Pyrolysis of Alkyd Paint Samples

Py - GC (see Fig. 5 for a typical pyrolysis - gas chromatogram) showed that the pyrolysis of this sample took place in a variable manner, and that the major pyrolysis products were acrolein, methacrolein, benzene and an unidentified component. The Py - MS results showed, in addition to these components, the presence of phthalic anhydride and benzoic acid. Table VI lists the results of the Py - GC experiments, and the Py - MS results are given in Tables VII–IX. For the Py - MS study 12 ions were monitored, with their intensities normalised to *m/e* 41 as 100%: *m/e* 39, 41, 55, 56, 57, 67, 70, 76, 78, 91, 104 and 105. Ions from this group appearing in the spectra of the major components are shown at the top of p. 343.

TABLE VI

PYROLYSIS - GAS CHROMATOGRAPHY OF ALKYD PAINT

Proportion of major components (relative to acrolein), relative standard deviations given in parentheses.

	Acrolein	Methacrolein	Benzene	Unidentified component
In-day variation (10 samples)	100	80.4 (5.6%)	41.8 (31%)	24.0 (5.3%)
Long-term variation (14 samples)	100	84.1 (7.4%)	28.0 (44%)	24.5 (6.6%)

Component	Mass spectrum (relevant ions only) *m/e* (%*I*)
Acrolein	56 (100), 55 (75)
Methacrolein	41 (100), 39 (70), 70 (70)
Benzene	78 (100)
Unidentified component	56 (100), 41 (80)
Benzoic acid	105 (100), 39 (11)
Phthalic anhydride	104 (100), 76 (85)

The ion at *m/e* 57 could have originated from a variety of hydrocarbon materials, that at *m/e* 67 from a variety of cyclic or unsaturated hydrocarbon compounds and that at *m/e* 91 could have come from a compound containing a benzyl group.

The results given in Tables VI–IX show that Py - GC does not monitor all the pyrolysis products; acrolein, methacrolein and the unidentified component are formed reasonably reproducibly, while benzene is formed very irreproducibly. No significant amounts of phthalic anhydride or benzoic acid are observed in Py - MS when using the Curie-point

TABLE VII

Py - MS OF ALKYD PAINT USING CURIE-POINT PYROLYSIS INTO GLASS TUBE

In-day variation: 10 samples, 12 spectra averaged for each run.

Ion (*m/e*)	39	41	55	56	57	67	70	76	78	91	104	105
Intensity	56.8	100	52.9	44.1	33.6	24.2	25.3	2.1	9.7	14.4	4.1	8.0
Relative standard deviation, % ..	6.3	—	2.6	1.3	4.6	3.1	1.7	43	18	3.9	43	13

FIT factors (45 comparisons) average 996, worst 988.

Long-term variation: 14 samples, 12 spectra averaged for each run.

Ion (*m/e*)	39	41	55	56	57	67	70	76	78	91	104	105
Intensity	53.0	100	54.0	49.3	36.0	22.6	29.7	—	10.9	13.2	5.2	7.0
Relative standard deviation, % ..	5.8	—	6.1	6.5	8.3	4.5	6.8	—	24	8.4	61	22

FIT factors (45 comparisons) average 994, worst 977.
Four samples were omitted from the FIT factor calculations as they suffered interference from the *m/e* 43 ion during background subtraction.

Long-term variation: 14 samples, single scan.

Ion (*m/e*)	39	41	55	56	57	67	70	76	78	91	104	105
Intensity	53.2	100	59.0	50.4	43.9	26.5	32.0	—	9.8	18.3	15.5	13.7
Relative standard deviation, % ..	7.5	—	8.9	8.4	11	4.5	7.8	—	23	13	51	23

TABLE VIII

Py - MS OF ALKYD PAINT USING CDS PYROPROBE PYROLYSIS INTO GLASS COLUMN

In-day variation: 10 samples, 12 spectra averaged for each run.

Ion (*m/e*)	39	41	55	56	57	67	70	76	78	91	104	105
Intensity	55.0	100	69.6	37.2	37.7	33.7	24.2	74.7	20.9	21.8	81.1	36.2
Relative standard deviation, % ..	3.7	—	3.8	3.5	5.7	3.9	3.6	10	16	4.1	10	8.0

FIT factors (45 comparisons) average 995, worst 983.

Long-term variation: 12 samples, 12 spectra averaged for each run.

Ion (*m/e*)	39	41	55	56	57	67	70	76	78	91	104	105
Intensity	58.6	100	62.0	38.9	33.7	32.4	25.0	58.4	18.6	20.4	61.5	21.3
Relative standard deviation, % ..	2.6	—	3.7	4.5	3.1	3.9	3.5	25	22	7.0	27	10

FIT factors (66 comparisons) average 978, worst 909.

Long-term variation: 12 samples, single scan.

Ion (*m/e*)	39	41	55	56	57	67	70	76	78	91	104	105
Intensity	59.7	100	61.7	37.4	32.0	33.5	25.3	55.9	24.4	23.2	59.0	23.4
Relative standard deviation, % ..	4.2	—	5.4	4.7	6.6	5.1	5.5	20	22	5.4	20	8.6

TABLE IX

Py - MS OF ALKYD PAINT USING VG PYROLYSIS PROBE

In-day variation: 9 samples, 52 spectra averaged for each run.

Ion (m/e)	39	41	55	56	57	67	70	76	78	91	104	105
Intensity	61.2	100	56.6	44.3	25.3	24.7	25.9	18.8	28.4	14.0	22.0	11.2
Relative standard deviation, %	4.0	—	4.9	3.0	2.5	7.0	3.6	13	17	2.8	9.5	4.3

FIT factors (36 comparisons) average 994, worst 981.

Long-term variation: 12 samples, 52 spectra averaged for each run.

Ion (m/e)	39	41	55	56	57	67	70	76	78	91	104	105
Intensity	57.7	100	61.9	45.5	34.2	22.6	27.4	44.7	9.3	11.4	47.9	13.3
Relative standard deviation, %	5.4	—	11	9.6	19	9.4	12	22	27	21	17	32

FIT factors (66 comparisons) average 976, worst 930.

Long-term variation: 12 samples, single scan.

Ion (m/e)	39	41	55	56	57	67	70	76	78	91	104	105
Intensity	55.7	100	59.5	44.8	30.5	23.0	27.5	26.9	12.2	12.4	29.6	12.1
Relative standard deviation, %	9.5	—	13	9.6	18	6.6	8.8	17	20	13	15	17

pyrolyser with the glass column. This is presumably caused by these components being deposited on the cold silica liner in the pyrolysis unit. The ions from the other pyrolysis products are present in greater proportions, but the variations in ion intensities are somewhat greater than the variations observed for the acrylic paint, reflecting the irreproducible pyrolysis of the alkyd paint. The over-all reproducibility, as measured by the FIT factors, is good, even though all possible information is not available for the FIT factor calculations.

All the pyrolysis products from the paint appear to be observed in Py - MS when using the CDS Pyroprobe. Good reproducibility is measured for some ions, although for other ions, especially those associated with benzene, phthalic anhydride and benzoic acid, the reproducibility is poor. The FIT factors show the over-all reproducibility to be similar to that obtained when using the Curie-point pyrolyser with the glass column.

The VG pyrolysis probe initially produced spectra similar to those from the Pyroprobe, but after work on pyrolysis - CI mass spectrometry using this probe, much less phthalic anhydride and benzoic acid was observed. This is caused, presumably, by carbon being deposited in the pyrolysis probe line. The long-term variation with this probe showed some irreproducibility, but this may be caused by line temperature variations that were observed with this prototype probe.

The variation in the proportion of benzene observed, as monitored by the m/e 78 ion, is smaller for the three Py - MS systems than for the Py - GC system. Possibly there is some contribution to the intensity of the m/e 78 peak from components that are produced more reproducibly than benzene.

Typical mass pyrograms for the alkyd paint samples on the three Py - MS systems are given in Fig. 6. FIT factors between these three mass pyrograms are less than 900.

Conclusions

The results obtained probably do not reflect the optimum Py - MS experimental situation as the VG pyrolysis probe was a prototype model, and some experimental difficulties were encountered in its use. Additionally, some disruption of the glass column occurred during the repeated changeovers from the Curie-point pyrolyser to the CDS Pyroprobe and this disruption probably degraded the measured reproducibility. The results, however, do give an indication of the kind of reproducibility to be expected from Py - MS and the relative merits of each of the pyrolysis systems examined. The conclusions can be summarised as follows.

1. The irreproducibility arising from the mass spectrometer fragmentation and detection, as measured by the variation of the intra-compound ion ratios, was no more than 2%, even over a period of several weeks. Although only one source was used, this was subjected to

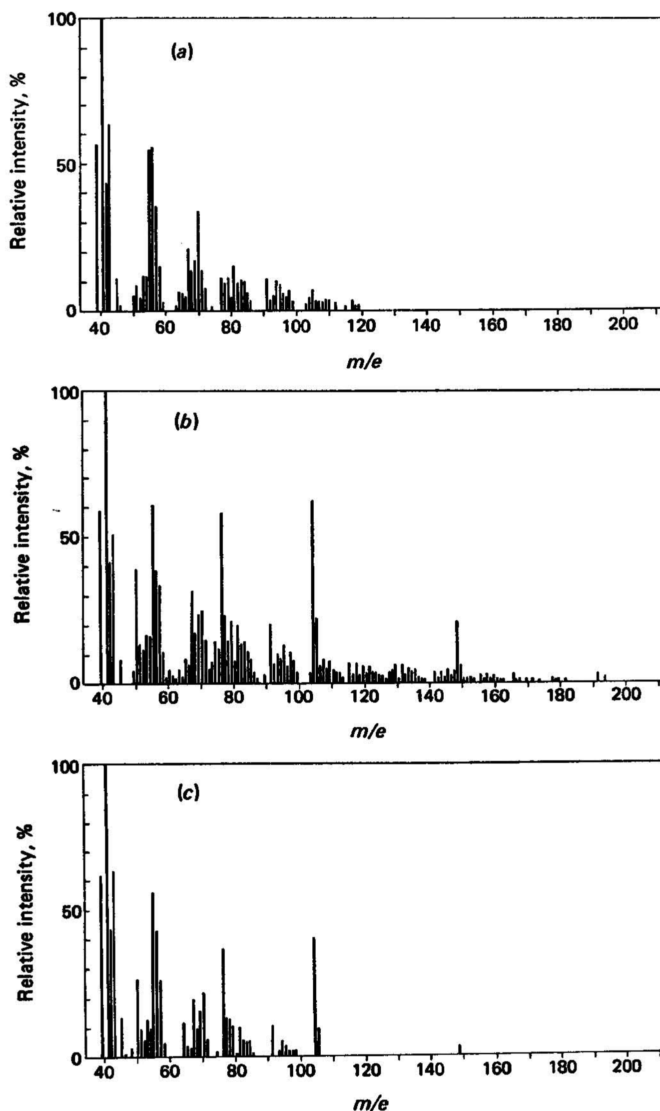

Fig. 6. Mass pyrograms for white alkyd paint. (*a*), Curie-point pyrolysis into glass column; (*b*), CDS Pyroprobe into glass column; and (*c*), VG pyrolysis probe.

a considerable amount of GC - MS analysis during this period. This shows that the mass spectrometric detection of a pyrolysate is unlikely to introduce large errors; the major factor in the irreproducibility of Py - MS will be the pyrolysis process.

2. As the EI fragmentation pattern is dependent on the instrumental tuning, it is recommended that the mass spectrometer is tuned to the spectrum of a reference compound, in order to achieve good instrumental reproducibility. Toluene spectra obtained on two other low-resolution magnetic sector mass spectrometers were very similar to the spectrum obtained with the present instrument. Hopefully pyrolysis spectra would also be similar on different instruments, but this will have to be tested by experiment. A study such as this would be necessary in order to determine whether an inter-laboratory exchange of data

is feasible; previous experience with Py - GC has shown that most laboratories build up in-house reference collections, making inter-laboratory reproducibility less important than intra-laboratory reproducibility.

3. Samples that pyrolyse simply and reproducibly, *e.g.*, the polymer-coated wires and the acrylic paint, gave good Py - MS reproducibility. If long-term reproducibility can be maintained, then for these types of substrate it would be feasible to compile a library system for identification and to employ FIT factors for quantitative comparisons.

4. The alkyd paint gave a more complex mass pyrogram than the acrylic paint (compare Figs. 4 and 6), and good reproducibility was observed only for some ions in its spectrum. FIT factors (using all ions) coupled with a library system would only be able to discriminate between alkyd paints of different chemical type. It may be possible to distinguish alkyd paints of a given type by selecting certain ions (*e.g.*, by discriminant analysis) for comparison, and this topic is currently under investigation.

5. For quantitative comparisons, Py - MS is more limited with respect to sample size, when compared with Py - GC; the Py - MS systems used would only operate satisfactorily over a relatively narrow range of sample mass.

6. Averaging several scans gave more reproducible results, compared with taking a single scan, for those ions that were already reasonably reproducible (approximately 5%). The reproducibility was not improved for those ions that showed large irreproducibility (>10%).

7. The best experimental results were obtained with the CDS Pyroprobe connected in line to a glass column, and with the VG pyrolysis probe.

The advantages of the CDS Pyroprobe are that it handles smaller samples better, the pyrolysate arrives in the mass spectrometer over a relatively short time interval and greater proportions of the less volatile components are produced during the pyrolysis. Disadvantages lie in the slowness of changing samples, the fact that the gas flows are interrupted during the sample introduction and that the pyrolysate enters the mass spectrometer through a restrictor and the jet separator.

Advantages in the use of the VG pyrolysis probe are that the Curie-point wires employed give a reproducible pyrolysis temperature, which is important for inter-laboratory comparisons. The probe is fairly quick in analysis time, and it is possible to mount several samples in the Curie-point wires before starting the experiment. Instrumentally, it is a simpler system, and the pyrolysate passes directly into the ionisation source. On the other hand, pyrolysate introduction takes place over a long period of time, and gives a lower absolute sensitivity, but some of the troubles encountered in its use may be ascribed to the probe being an experimental model.

We are grateful to the Home Office Central Research Establishment, Aldermaston, for the loan of the VG pyrolysis probe. We thank Dr. M. J. Whitehouse for useful advice on all aspects of mass spectrometry.

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Received *August* 30*th*, 1978
Accepted *October* 18*th*, 1978

 Analyst, April, 1979, Vol. 104, pp. 348–357

Interference Films on the Sensor Membranes of Solid-state Copper(II) Ion-selective Electrodes

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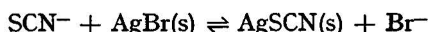
Copper(II) ion-selective electrodes based on copper(II) sulphide - silver sulphide sensor membranes, which showed anomalous responses with copper(II) nitrate in the presence of chloride, have been examined by Auger spectrometry. In some electrodes exposed to solutions of potassium chloride the chloride is found to have penetrated the bulk of the membrane matrix, whilst in others only a surface contamination is observed. The anomalous electrode response is exhibited when exposure to chloride is in the presence of copper nitrate. The Auger signal alters during the duration of the spectrum as a consequence of electron bombardment. The effects of argon-ion and electron bombardment are compared.

Keywords: *Ion-selective electrodes for copper(II); interference films on ion-selective electrodes; Auger spectrometry*

A common type of interference encountered with solid-state ion-selective electrodes[1] involves interaction between the interferent and sensor membrane component(s) to form new compound(s) of varying degrees of solubility. Thus, the Orion 94-35 bromide electrode can tolerate thiocyanate to a level dictated in principle by the quotient of the respective silver salt solubility products, $K_{SO_{AgSCN}}/K_{SO_{AgBr}} \approx 3$. Beyond this value the silver thiocyanate film formed on the surface of the silver sulphide - silver bromide sensor solicits a near-Nernstian response to thiocyanate



$$SCN^- + AgBr(s) \rightleftharpoons AgSCN(s) + Br^-$$

Fortunately, no permanent damage is incurred and the offending film is removed with emery cloth when the "thiocyanate" electrode reverts to its original bromide-sensing role.[1] However, interference from iodide ions can be considerably less than would be predicted on the basis of solubility product ratios according to the extent of the interference deposited on the sensor surface of a chloride ion-selective electrode.[2]

An interesting effect is observed with the Orion 94-29A copper solid-state ion-selective electrode immersed in copper(II) nitrate solution. Chloride leakage from a calomel reference electrode, or deliberate addition of the chloride ion, rapidly dulls the normal shiny sensor surface and the electrode gives a chloride, not copper, ion activity response. Nevertheless, the original copper function is completely restored after polishing about half of the dulled sensor surface. This copper to chloride to copper response cycle has been repeated many times and with different Orion 94-29A electrodes.[3]

The chemical nature of this chloride-responsive electrode film remains unidentified, but it may consist of a chloride species arising by, say

$$Ag_2S(s) + Cu^{2+}(aq) + 2Cl^- \rightleftharpoons CuS(s) + 2AgCl(s)$$

Preliminary attempts to identify chloride complexes in simulated silver sulphide - copper(II) sulphide materials were unsuccessful.[3] Therefore, a variety of copper(II) sulphide - silver sulphide - potassium tetrachlorocuprate(II) discs have been exposed to potassium chloride or copper(II) nitrate - potassium chloride solutions and their surfaces examined by Auger spectroscopy[4] in a further attempt to establish the nature of the interference. In addition, the potentiometric behaviour of some selected discs has been measured before and after such

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chemical exposures (Table I) but, owing to the fragile nature of the discs, it was not possible to examine their potentiometric behaviour after Auger examination.

TABLE I

COMPOSITION AND TREATMENT OF ELECTRODE DISCS PRIOR TO AUGER* EXAMINATION

Disc series	Disc composition (molar ratio)	Soaking treatment	Slope, S, (in mV per decade) and lower linear calibration range (R) of electrode in standard solutions at 25 °C	
			After soaking	Before soaking
Orion 94-29 electrode	$CuS:Ag_2S$ (2:3)†	KCl (1.3 M) for 1 week	No Cl^- response in KCl S = 31.0; R = 10^{-5} M in $Cu(NO_3)_2$	S = 30.8; R = 10^{-5} M in $Cu(NO_3)_2$ S = 28.0; R = 10^{-6} M in $CuSO_4$
		20 cm³ $Cu(NO_3)_2$ (10^{-1} M) + 0.5 cm³ KCl (1.33 M) for 10 min	No Cu^{2+} response in $Cu(NO_3)_2$ or $CuSO_4$ Cl^- response in both KCl and $CuCl_2$ standards. S∼61; R∼10^{-4} M	S = 28.5; R = 10^{-6} M in $Cu(ClO_4)_2$ S = 27.0; R = 10^{-5} M in $Cu(OAc)_2$
A	$CuS:Ag_2S$ (1:1)	KCl (1.3 M) for 5 min	S = 29.8; R = 2×10^{-5} M in $Cu(NO_3)_2$	S = 29.8; R = 2×10^{-5} M in $Cu(NO_3)_2$
B	$CuS:Ag_2S$ (1:1)	Control	—	
C	$CuS:Ag_2S$ (1:1)	20 cm³ $Cu(NO_3)_2$ (10^{-1} M) + 0.5 cm³ KCl (4 M) for 10 min	No Cu^{2+} response below 10^{-3} M $Cu(NO_3)_2$ but S = 25 mV above 10^{-3} M $Cu(NO_3)_2$ No Cl^- response in KCl and $CuCl_2$ standards Cu^{2+} response restored after polishing membrane	S = 30.0; R = 2×10^{-5} M in $Cu(NO_3)_2$
D	$CuS:Ag_2S$ (1:9)	KCl (1.3 M) for 5 min	S = 30.3; R = 5×10^{-5} M in $Cu(NO_3)_2$	S = 30.3; R = 5×10^{-5} M in $Cu(NO_3)_2$
E	$CuS:Ag_2S$ (1:9) + $K_2[CuCl_4]$ (1% *m/m*)	KCl (1.3 M) for 5 min	S = 10; R = 10^{-4} M in $Cu(NO_3)_2$	S = 12; R = 10^{-5} M in $Cu(NO_3)_2$ No Cl^- response
F	$CuS:Ag_2S$ (1:1) + $K_2[CuCl_4]$ (1% *m/m*)	KCl (1.3 M) for 5 min	S = 15; R = 10^{-4} M in $Cu(NO_3)_2$	S = 14; R = 10^{-4} M in $Cu(NO_3)_2$. No Cl^- response

* Auger examination not feasible with the whole electrode assembly.
† See Frant and Ross.[5]

Experimental

Electrodes

Discs of various compositions were prepared by pressing[3] from mixed metal sulphides after co-precipitation from solution and very thorough washing with de-ionised water. Electrodes were then assembled from hollow poly(vinyl chloride) (PVC) bodies with the sensor discs sealed into one end, using the epoxy resin cement, Araldite.[6] An internal silver - silver chloride reference electrode immersed in a copper(II) nitrate (10^{-1} M) and potassium chloride (10^{-3} M) internal filling solution was then sealed in at the other end of the PVC stem. With only copper chloride as the internal solution calibration graph slopes were less than 20 mV per decade. All of the electrodes were calibrated in solutions of copper(II) sulphate, copper(II) acetate, copper perchlorate, copper(II) nitrate or potassium chloride, as appropriate, in conjunction with an Orion double-junction reference electrode containing 4 M potassium nitrate solution. The potentials of these cells were then recorded with a Beckman 4500 digital pH meter by using various test solutions kept at 25 ± 0.1 °C.

Auger Electron Spectroscopy (AES)

Discs for AES were treated as in column 3 of Table I, drained, air-dried and examined on a retarding field analyser Auger spectrometer made by Vacuum Generators, Sussex. The spectrometer was supplemented by an additional electronic device controlling the potentials of electrostatic plates, thus ensuring convenient deflection and improved focusing of the primary electron beam. The observed energies of the emerging electrons were displayed by an electronic digital voltmeter and the Auger spectra drawn by an *X–Y* plotter (Advance-HR-2000). The pressure in the analyser was about 1.3×10^{-8} Pa (10^{-10} Torr).

The specimen was bombarded with 1.1-keV electrons at an angle of incidence of about 15°, the beam of the primary electrons being about 1 mm in diameter. The spot of the specimen surface hit by the electrons showed, therefore, a nearly elliptical shape with axis lengths of 4 and 1 mm. The primary beam current was about 70 μA and the sample bias 90 V.

Spectra were normally taken in the 0–400 eV energy range (some up to 1000 eV) and repeated several times at different modulating voltages (RMS). The sweep time applied was usually 1000 s per scan, that is, 0.4 eV s^{-1}.

The focusing of the incident electron beam was carried out by maximising the intensity of the 272-eV carbon signal. Neither the position of the specimen nor the electrical settings were changed in the course of the investigation of a given sample. The Auger transitions used are listed in Table II. Representative spectra are shown in Figs. 1–3. The copper peak at 63 eV was not clearly resolved in many of the spectra because of the electron-energy analyser incorporated in the spectrometer. Consequently, no quantitative measurements of copper were attempted.

TABLE II

AUGER SIGNALS CHOSEN FOR THE ELEMENTS[4]

Element	Z	Nominal energy/eV	Auger transition
Cu	29	63	$M_3M_4M_4$
S	16	152	$L_3M_{2,3}M_{2,3}$
Cl	17	181	$L_3M_{2,3}M_{2,3}$
K	19	252	$L_3M_{2,3}M_{2,3}$
C	6	272	KL_2L_2
Ag	47	304	$M_4N_{2,3}M_{4,5}$
Ag	47	356	$M_5N_{4,5}N_{4,5}$
O	8	510	KL_2L_2

The carbon peak is a well known problem in surface-sensitive techniques and arises principally from surface adsorbed carbon dioxide and, possibly, diffusion pump oil vapour. It is usually removed during the early stages of argon-ion bombardment. Persistence of carbon through a series of bombardments (for example, Fig. 6) may therefore be taken to indicate the presence of carbon in the sample, for example, in a carbonate impurity. However, it is not possible to draw firm qualitative or quantitative conclusions from the available evidence.

Argon-ion bombardment (Fig. 2) for semi-quantitative depth profiling was performed at argon pressures of about 1.3 × 10^{-2} Pa (10^{-4} Torr). The source of the argon ions was the ionisation gauge, built-in for pressure measurements. The usual bombarding current was 20–30 μA. In the initial work the specimen was turned to face the ion gun in order to achieve a perpendicular ion impact; later, however, its position was fixed at an incident angle of about 20°. The bombardment period (5–10 min) was repeated 8–10 times.

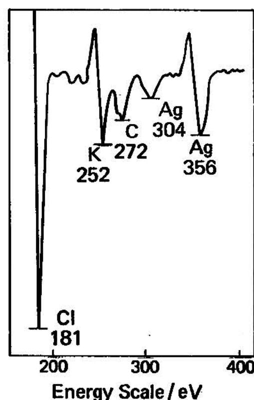

Fig. 1. Representative Auger spectrum of disc A with hand-drawn energy scale. Scan speed, 0.4 eV s^{-1}; RMS, 3 V.

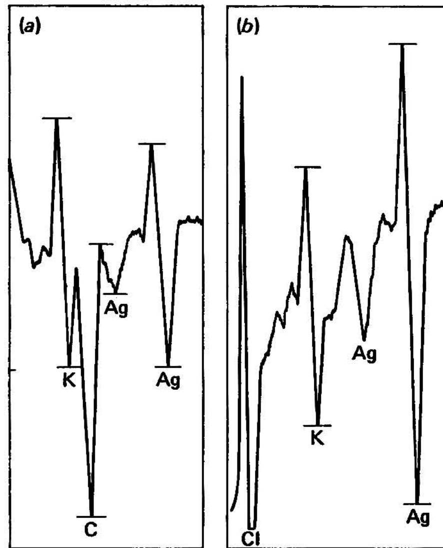

Fig. 2. Auger spectra of disc A with hand-drawn scales. (*a*), Before argon-ion bombardment; (*b*), after 740 μA min argon ion bombardment. Scan speed, 0.4 eV s^{-1}; RMS 4 V.

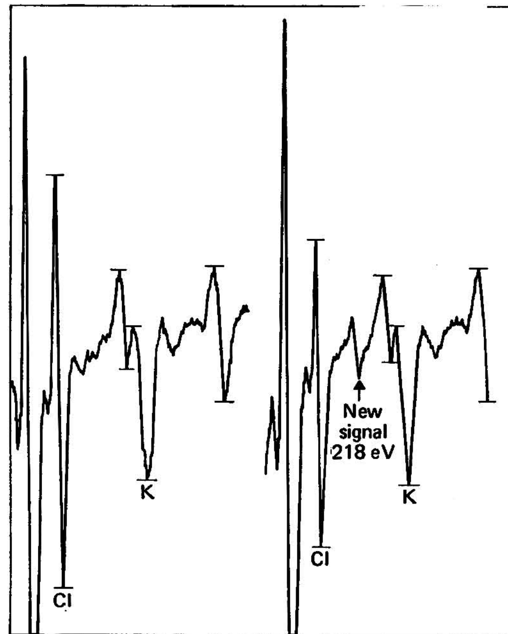

Fig. 3. Auger spectra of disc A with hand-drawn scales showing evidence of a new peak at 218 eV attributed to trapped argon (between the Cl and K signals) in the later electron irradiation exposure of the right-hand spectrum. Scan speed, 0.4 eV s^{-1}; RMS, 4 V.

Results and Discussion

Auger Spectroscopy

The Auger signal is proportional to the amount of the element from which it is derived but the proportionality factor depends on the element and the particular transition. In this work the changes in signal are taken to indicate changes in element concentration but no attempt has been made to relate this numerically to the actual percentage chemical composition. As a guide, an argon-ion etching of 1 μA min corresponds roughly to the removal of 0.1 nm of material. However, in these samples the combined effects of electron bombardment by the source and argon-ion etching make the application of simple guide lines difficult.

Effects of electron bombardment

In electron-bombardment examinations of an electrode disc (A in Table I) by AES, the same spot on the sample was repeatedly scanned and all signal intensities changed with time (Figs. 1 and 4). The signals for chloride and potassium decreased at unequal rates (Fig. 4), while carbon and silver showed more marginal changes (Fig. 4).

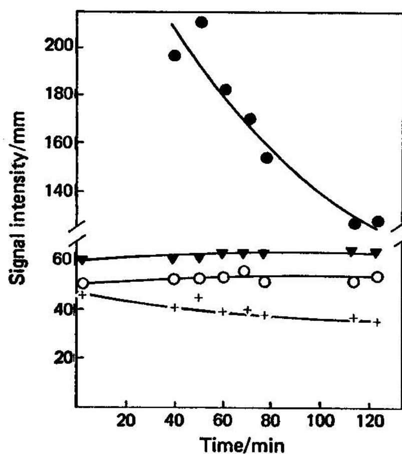

Fig. 4. Summarising diagram of changes of Auger signal intensities with time for disc A on electron bombardment. Scan speed, 0.4 eV s^{-1}; RMS, 3 V. ○, Silver; ●, chlorine; ▼, carbon; and +, potassium.

Any vacuum effects, for example, evaporation or sublimation, were discounted because a sample does not necessarily sputter evenly and because different spots on the same disc gave similar patterns.

The huge increase in the sulphur signal can cause overloading and for this reason it is not shown in all of the figures. However, the general shape of the overloaded sulphur peaks indicated substantial increases in the sulphur signals.

Clearly, the surface undergoes alterations as a result of electron irradiation during the time of recording the Auger spectrum. The resultant pitting of the surface was confirmed by a scanning electron microscope. Hence, AES was supplemented by argon-ion bombardment.

Effect of argon-ion bombardment

The usual practice, in Auger spectroscopy, for removing surface contaminants is to fracture the specimen, scrape its surface in a very low vacuum or to sputter the surface with highly energetic ions of noble gases. This last method is likewise applicable to surface depth

profiling, as noble gas ions hitting the surface with appropriate kinetic energy are able to remove surface layers many hundred Ångstroms in thickness, which is at least ten-fold greater than the thickness of the layers effectively analysed by the electron beam.

In this work, argon-ion sputtering was used and the following points were qualitatively concluded for disc A: that the surface layer of carbon is removed during sputtering; that sputtering tends to "restore" the surface and slightly moderate the changes induced by electron bombardment (effects that lie on the border between significant and insignificant); after each sputtering, that is, on each newly exposed surface, electrons affect the specimen in the same way as before; and that changes induced by electrons in various scans are comparable to those caused by ion bombardment.

These conclusions are illustrated for the combined effects of electron and argon-ion bombardment for disc A in Figs. 5 and 7 and disc D in Fig. 6. It will be noted that despite the effects of electron bombardment there are over-all trends that are made evident by argon-ion bombardment and these are shown in Fig. 8. Some general results are presented in Table III. These are obviously tempered by the considerations given above, but are taken to refer to profiles from 0.01 to 10 μm. It should be noted that the ratios in the ordinates of Figs. 5, 6 and 8 relate to signal intensities and not to actual chemical composition.

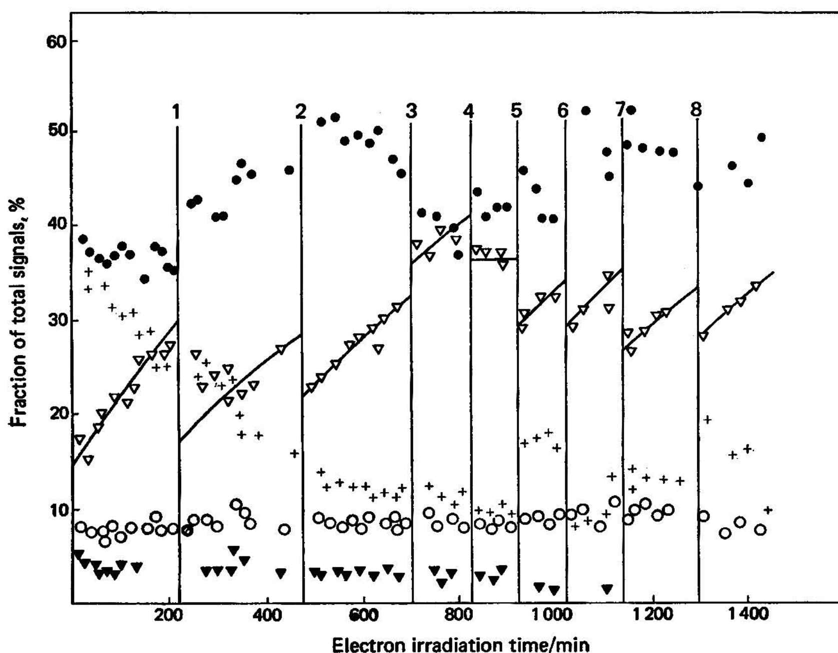

Fig. 5. Changes, for disc A, with electron irradiation time interspersed by argon-ion bombardments of contributions of individual signals expressed as percentages of total of signals for the various species. ●, Chlorine; ○, silver; +, potassium; ▽, sulphur; ▼, carbon.

From the above it can be deduced that both potassium and chloride ions have penetrated the membrane (A) to an extent approaching 0.1 μm. This distance can be estimated on the basis of removing 0.1-nm layers for each 1 μA min of argon-ion bombardment. However, in view of the changes that can take place in the sample consequent on bombardment, the calculations are, at best, approximate.[7,8]

In principle, Auger spectroscopy can distinguish oxidation states of elements, but the resolution of the present instrument was unfortunately too low for this purpose. The results for the several discs examined are summarised in Table IV.

Potentiometric Measurements and Their Relationship to AES Results

The potentiometric performance of electrodes with sensor discs composed of copper(II) sulphide - silver sulphide in a 1:1 molar ratio (discs A, B and C) compared favourably with

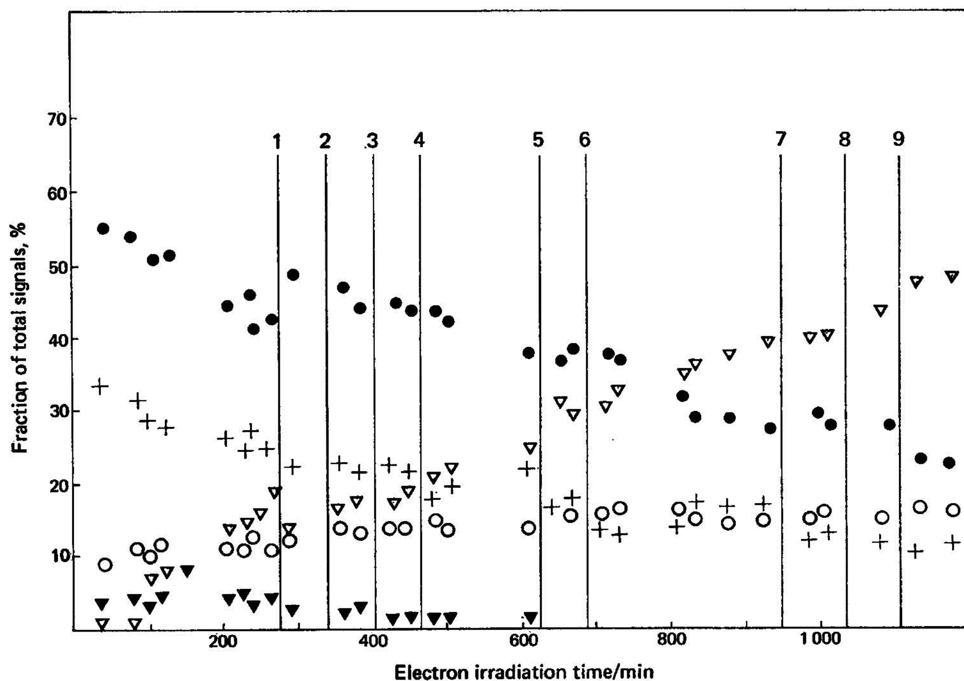

Fig. 6. Changes, for disc D, with electron irradiation time interspersed by argon-ion bombardments of contributions of individual signals expressed as percentages of total of signals for the various species. ●, Chlorine; ○, silver; +, potassium; ▽, sulphur; and ▼, carbon.

the commercial Orion 94-29 electrode (Table I). Disc D, with a 1:9 molar ratio of copper(II) sulphide and silver sulphide, behaved similarly (Table I), but sensor discs of 9:1 molar ratio of copper(II) sulphide and silver sulphide were too fragile to be useful for electrode con-

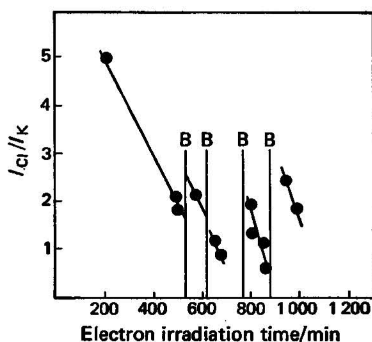

Fig. 7. Change of ratio of chlorine: potassium signal intensities with time for disc A on both argon-ion and electron bombardment. The vertical lines indicate argon ion bombardment. μA min: (1) 130; (2) 200; (3) 208; (4) 200.

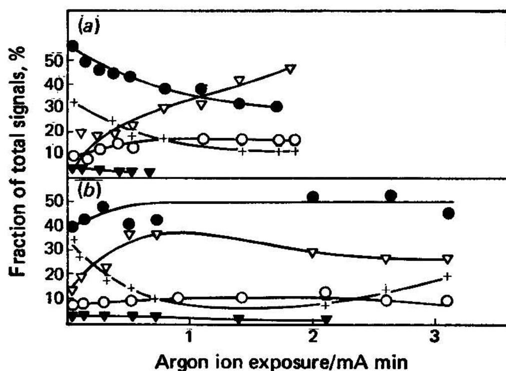

Fig. 8. Effect of argon-ion bombardment on composition of discs A and D. The values correspond to the first spectrum obtained after the bombardment and are normalised to percentages of total K, S, Cl, C and Ag signals. (*a*), Disc D; and (*b*), disc A. ●, Chlorine; ○, silver; +, potassium; ▽, sulphur; and ▼, carbon.

struction and in any case responded poorly to copper standards. This fact illustrates the importance of incorporating sufficient silver sulphide into the sensor matrices of many solid-state ion-selective electrodes.[9] In addition, the resistivity of the discs (measured with a Multimeter) is a function of the silver sulphide content as observed for silver chloride - silver sulphide[9] and silver bromide - silver sulphide[6] sensor discs.

TABLE III

EFFECT OF ARGON ION BOMBARDMENT ON ELECTRODE DISC A

Time of electron irradiation/min	RMS voltage	Signal intensities/mm Cl	K	C	I_{Cl}/I_K	Comment
214	2	100.5	20.5	10.5	4.9	
490	2	28.8	14.5	10.8	1.99	
511	4	136	79.2	61	1.72	
567	2	31.2	1.46	11.5	2.14	After 130 μA min Ar^+
649	2	15	13	TS*	1.15	After a further 200 μA min Ar^+
668	4	63.1	71	9	0.89	
802	2	20	10.8	TS*	1.85	Refocus, further 208 μA min Ar^+ bombardment
814	4	83.8	64.4	17.7	1.30	
846	4	65.5	61.2	16.1	1.07	Focus on Ag signal
864	4	48.6	81.8	TS*	0.59	
948	2	44.2	18.5	0	2.39	After further 200 μA min Ar^+ bombardment
988	3	97.4	52.2	0	1.87	

* TS = Too small to measure.

Electrodes with discs (E and F) containing 1% *m/m* of potassium tetrachlorocuprate(II) dihydrate respond poorly to copper(II) nitrate. The possibility that this complex may be related to the primary interferent species must be tempered by the fact that electrodes incorporating it do not respond to chloride (Table I). Also, infrared spectroscopic examination (200–4000 cm^{-1}) of this finely ground disc material in potassium bromide matrices showed no definite evidence of copper chloride complexes. Auger spectroscopy could not provide evidence on this matter either and there was, in addition, an inability to detect potassium owing to overlapping carbon signals (Table IV).

The response of various copper(II) ion-selective electrodes is modified by fluoride and chloride to an extent dependent on age, condition of membrane surface, the presence of

acids and their associated anions, oxygen tension and exposure times.[10] The absence of short-term chloride effects for new discs (discs A and D) has also been found for freshly polished membranes.[10]

Significant chloride interference arises only when potassium chloride is added to copper(II) nitrate solutions in which the Orion 94-29 electrode or the models fitted with various pressed silver sulphide - copper(II) sulphide discs are immersed or when these electrodes are immersed in copper(II) nitrate - potassium chloride solutions. This behaviour is apparent from the results given in Table I for the Orion 94-29 electrode and that made from disc C, for which the Auger spectroscopic data are given in Table IV. Similar behaviour was also observed for an electrode made from a disc of copper(II) sulphide - silver sulphide (1:9) and soaked in 20 cm^3 of copper(II) nitrate solution (10^{-1} M) and 0.5 cm^3 of potassium chloride solution (4 M). The presence of copper(II) nitrate in some way sensitises the membrane to interference from chloride. Auger spectroscopy can confirm this insofar as nitrate is present in the membrane matrix for disc C.

TABLE IV

RESULTS OF AUGER SPECTROSCOPIC EXAMINATION OF VARIOUS SENSOR DISCS

Disc series	Comments on electron and argon-ion impact experiments
A	Sulphide signals increase with depth. Potassium and chloride ions penetrate the membrane to an extent approaching 100 nm
B	Reference disc: evidence of copper, silver, sulphide and carbon only
C	Steady pattern with depth, showing evidence of silver, copper, potassium, sulphide and chloride ions. Nitrogen (from nitrate) is observable and constant after a 250 μA min argon-ion bombardment
D	Little evidence of sulphide at the surface where potassium and chloride are predominant. Sulphide increases with depth while chloride and potassium decrease
E	Evidence for sulphide constant with depth. Potassium was not detected, possibly because of overlapping carbon signal. Chloride, silver and carbon remain constant with depth
F	Sulphide increases with depth, while chloride falls. Potassium not detected, possibly because of overlapping carbon signal. Silver and carbon levels remain constant with depth

There is no clue to the operative mechanism, although it probably involves copper in conjunction with chloride, as soaking the Orion 94-29 electrode, or electrodes made with pressed membranes of copper(II) sulphide - silver sulphide (1:1), in potassium chloride solution alone or in potassium chloride plus potassium nitrate solution does not affect calibration with copper(II) nitrate. On the other hand, the observation noted above, concerning the more rapid decrease in the chloride signal in comparison with that for potassium during electron and argon-ion bombardments of disc A, suggests that some complexation of chloride takes place in the surface disc layers. This may be upset by the presence of copper(II) nitrate, although the observed patterns neither confirm nor discount this.

The massive rise in the Auger sulphur signal with depth (Table IV) could be caused partly by surface sulphur arising from membrane oxidation,[11] which either diffuses away or is reflected in a relatively weaker elemental sulphur signal.

$$CuS(s) + \tfrac{1}{2}O_2 + 2H_3O^+ \rightarrow Cu^{2+}(aq) + 3H_2O + S(s)$$

Unfortunately, AES cannot distinguish sulphur from sulphide in this work. Also, there is no evidence of the e.m.f. values of the copper ion selective - reference electrodes system changing in a positive direction, as would be expected from a release of copper ions.

Conclusion

The study of surface layers of ion-selective electrode discs of copper(II) sulphide and silver sulphide by use of Auger spectrometry, involving argon-ion and electron bombardment, shows that chloride penetration of the membrane disc occurs. This phenomenon

can be responsible for interference with the copper ion-selective electrodes by chloride, although the presence of copper(II) nitrate appears to be necessary for stimulating such interferences.

The authors thank the University of Technology, Baghdad, Iraq, for paid leave of absence (to N. S. N.), The Royal Society for a Visiting Fellowship (to P. S.), the University of Wales for a Studentship (to B. J. W.), Mrs. P. Connors for technical assistance and M. Jones (Department of Mechanical Engineering, University College, Swansea) for Auger spectrometer facilities.

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Received *August 21st*, 1978
Accepted *November 2nd*, 1978

Analyst, April, 1979, Vol. 104, pp. 358–366

Determination of Nitrite Ion in Unused Cutting Fluids and Cutting Oils Using a Gas-sensing Electrode Method

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Modifications of the Orion NO_x gas-sensing electrode method that were made to determine nitrite ion in unused cutting fluids and cutting oils are described. Detection limits for both types of lubricants of the order of 15 $\mu g\, g^{-1}$ of NO_2^- ion were obtained. Previous analysis of six cutting fluids, collected in the Ottawa region and analysed by spectrophotometry, confirmed the presence of high levels of nitrite ion and showed fair agreement between results. Analysis of six other cutting fluids and 20 cutting oils collected in the same region showed the presence of nitrite ion in only three instances. The operation of the electrode, interferences, the use of standard-addition and -subtraction methods and the possibility of applying this method to used cutting lubricants are discussed.

Keywords: *Nitrite-ion determination*; *gas-sensing electrode*; *nitrogen oxide electrode*; *cutting fluids*; *cutting oils*

Metal-working lubricants are used in cutting, grinding, rolling and drawing operations in many industrial and trade applications.[1,2] They are generally referred to as oils if they contain a hydrocarbon base (either a petroleum fraction or animal fat) and fluids or solutions if they are synthetic chemical solutions without a hydrocarbon base. Semi-synthetic lubricants are mixtures of oils and fluids. The oil-type lubricants can be made to mix with water by addition of a suitable emulsifier, or can be left non-emulsifiable. Fluid-type lubricants are usually water soluble. All of these products are available in Canada and some typical formulations have been compiled.[2,3]

The presence of nitrite salts, added to cutting fluids as rust inhibitors (and therefore present in semi-synthetic cutting oils), has recently become of interest to health regulatory and consumer protection agencies owing to the reaction between nitrite ion (or nitrous acid) and the common components, diethanolamine and triethanolamine (acting as either rust inhibitors or as part of the emulsifying agent) to form *N*-nitrosodiethanolamine,[4,5] regarded as one of the most potent of animal carcinogens. Danger to both the animal and human organism has been reviewed in detail[6,7] and, indeed, deaths in China have been attributed to this compound.[8] In the Ottawa region, up to 45% of triethanolamine, 18% of sodium nitrite and 3% of *N*-nitrosodiethanolamine have been found in cutting fluids,[9,10] levels that are considered to present a real danger to machinists through skin contact and mist inhalation.

Other precursors can participate in the formation of *N*-nitrosamines under widely varying conditions.[6,11,12]

While nitrite salts have been used as rust inhibitors in cutting fluids, nitrites have not been added to non-emulsifiable lubricants. In addition, most companies faced with the possibility of legislation in the USA and Canada have removed nitrites from lubricant formulations or have removed the product from the market. Ethanolamine emulsifiers, however, have been retained.

This laboratory was charged recently with the analysis of unused cutting oils (this term will subsequently refer to water-emulsifiable cutting oils) and cutting fluids available in Canada for *N*-nitrosodiethanolamine. As this material may appear some time after mixing the precursors,[9,10] one method of control considered was the prohibition of the simultaneous presence of nitrite ion and di- or triethanolamine. Sensitive methods of detection of these

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precursors were sought because their safe concentration in cutting oils or fluids is not known, but is thought to be very low. The methods considered for nitrite-ion determination were restricted to those for free aqueous nitrite ion, or nitrite ion bound sufficiently weakly to other agents such that a simple room-temperature separation method would release it for analysis.

Although spectrophotometric methods are sensitive (detection limits are in the low micrograms or nanograms per millilitre range), numerous,[13–16] and are used as the basis of several standard methods,[17–20] it was envisaged that the water-soluble or micellar material in cutting oils could lead to spectral interference, unless significant dilutions of the sample were made; hence spectrophotometric methods were considered inadequate. Ion-exchange liquid chromatographic methods using either conductimetric[21,22] or ion-selective electrode[23] detection, capable of measuring concentrations down to 5 and 46 μg ml^{-1} of nitrite ion, respectively, were not investigated because the detection limits were too high. While the indirect, differential pulse-polarographic method reported by Boese *et al.*[24] can optimally detect 0.046 μg ml^{-1} of nitrite ion, there was no reason to believe that the probability of encountering interfering electroactive compounds in cutting fluids or oils would be low and, further, the method of standard additions is not possible because of curvature of the calibration graph. Although a calorimetric method was reported by Hansen *et al.*[25] to be highly selective, the sensitivity (detection limit about 69 μg ml^{-1} of nitrite ion) was not adequate.

On the other hand, nitrogen oxide (NO_x) gas-sensing electrodes (*e.g.*, Orion, Model 95-46) are economical, can detect concentrations of nitrite ion between 0.046 and 460 μg ml^{-1} and have analysis times comparable to those of the above methods. Additionally, the Orion electrode method has been shown to give increased precision and accuracy over two standard spectrophotometric methods, and not to respond to colour, turbidity and many diverse ions.[26–28]

The subject of this paper is the adaptation of the Orion electrode method to the quantitative analysis of unused cutting fluids and cutting oils for labile nitrite ion. Results of the analysis of several fluids and oils are presented, together with a summary of the electrode operation and interferences. Also, the use of standard-addition or -subtraction methods is discussed.

Experimental

Instrumentation

An Orion NO_x electrode (Model 95-46), a digital pH meter (Fisher Accumet, Model 520) and a strip-chart recorder (Perkin-Elmer, Model 56) were used to make all measurements. A magnetic stirrer (Cole-Parmer Spin-Master, Model 4803), adjustable micropipette (Pipetman, 0–200 μl) and centrifuge (Daman/IEC, Model UV) were also used. The Merckoquant nitrite-test papers were obtained from BDH (M10057-01).

Reagents

All chemicals and solvents were of reagent grade except where indicated otherwise. A 0.1 M sodium nitrite stock solution (Orion, number 95-46-08) was refrigerated at all times. Daily, a 10^{-3} M sodium nitrite working solution was prepared by accurately diluting 1 ml of the stock solution to 100 ml with distilled water. The acid - sulphate buffer was prepared as directed by the Orion manual.[26]

Methods

Calibration graphs were prepared by adding appropriate micro-aliquots of the working solution to 50 ml of buffered distilled water.

Analysis of water-diluted cutting fluids (diluted to about 300 *V*/*m*) was performed by adding about 0.2 g of potassium dichromate and then applying the modified Orion electrode method described below.

Analysis of cutting oils was performed as follows. The cutting oil, weighed accurately (0.2–0.4 g), was dissolved in 100 ml of practical-grade 2,2,4-trimethylpentane (Fisher P-2396). Saturated calcium sulphate solution (100 ml) was added and the mixture was agitated for 30 s; the phases were then allowed to separate for a minimum of 1 h. The

aqueous phase was isolated and residual organic matter was separated from it by centrifugation at 16 s^{-1} for 600 s. To 45 ml of aqueous phase, about 0.2 g of potassium dichromate was added and the Orion electrode method[26] applied. After the electrode response had equilibrated, the potential was recorded and 100 μl of 1% sulphamic acid solution (Fisher A-295) were added to remove the nitrous acid selectively and quantitatively. After the electrode response had re-equilibrated, the potential was recorded again. The difference in the concentration, determined by comparison with a calibration graph before and after addition of sulphamic acid, was attributed to nitrite ion. The calibration graph was determined by analysing aqueous standards before and after analysing the set of samples.

When cutting oils were examined, the membrane was replaced after immersion for 45 min. Much longer exposure to water-diluted cutting fluids was possible.

Results and Discussion

Operation of NO_x Electrode

Various aspects of gas-sensing electrodes, including the NO_x type, can be found in the literature. These include the theory of operation and construction,[26–28] the possibility of increasing the sensitivity (with the ammonia electrode) by using a more dilute inner filling solution (IFS),[29] the rates of loss of nitrous acid or nitrite ion from standard solutions[30] and the extent of interferences from various diverse species affecting the Orion NO_x electrode.[26,31,32]

In summary, the response of the NO_x electrode obeys the Nernstian-like equation

$$E_{cell} = E_0 + S \log\left([NO_2^-] + \sum_i K_x[X_i]\right) \quad \ldots \quad (1)$$

where X_i represents an interfering species and K_x is its selectivity constant.

During this work, the linear range of the Orion NO_x electrode (Fig. 1) appeared to be between 0.23 and 230 $\mu g\ ml^{-1}$ (or 5×10^{-6} and 5×10^{-3} M, respectively) of nitrite ion with curvature at the lower end owing to dissolved carbon dioxide, according to the Orion method manual.[26] Using the same bottle of IFS and batch of membranes, the average slope and intercept obtained (Table I) were significantly different from those reported by Orion.[26] Each slope and intercept shown in Table I was determined by simple linear regression of the data into equation (1).

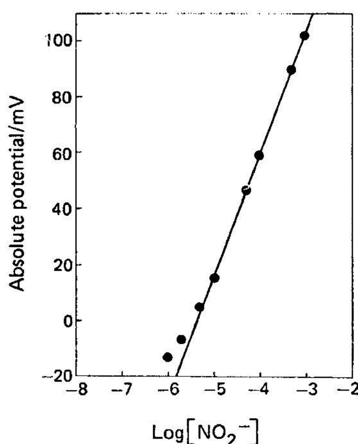

Fig. 1. Typical calibration graph using the Orion NO_x gas-sensing electrode.

The time required for electrode-potential equilibration was, on average, longer than that reported by Orion. About 4–12 min were required, depending on the membrane used, the magnitude and direction of concentration change and the sample type. In general, longer times were required with the metal-working lubricants analysed during this work.

*The reason for the apparent discrepancy of the slope value (43.5 mV) that is reported on p.361 and discussed on p.360 was probably due to a misadjusted or malfunctioning slope adjuster on the pH meter. Measurement of the slope well after the paper was published yielded values between 55 and 58 mV. This situation does not affect the results of the paper as the slope was usually measured on the same days that the results were generated

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TABLE I

TYPICAL SLOPE (S) AND INTERCEPT (K_0) VALUES OBTAINED USING THE ORION NO_x ELECTRODE

	Slope/mV	Intercept/mV
	45.8	233
	43.1	228
	41.0	229
	43.1	232
	47.4	245
	41.0	227
	42.8	231
Mean	43.5	232
Standard deviation	2.4	6

With respect to this work, interfering species could be described under three general categories: those which reacted with nitrous acid or nitrite ion in the sample before immersion of the electrode, those which dissolved the membrane or removed its hydrophobic activity (commonly called wetting) and those which diffused across the membrane to alter the pH of the IFS.

Oxidants (*e.g.*, hydrogen peroxide) and reductants (*e.g.*, sulphamic acid) are examples of the first type. This type of interference was observed during this work when several nitrite-free cutting oils were spiked with sodium nitrite solution during analysis and the electrode response was seen to increase for about 20 s at first and then to decrease quickly as the nitrous acid was removed (it is possible that sulphonate salts caused this effect). The presence of this type of interference did not pose any problem for this laboratory but ensured the absence of nitrite ion in the lubricants.

Micellar or water-soluble organic material, examples of the second type of interference, effected a serious restriction on the over-all sensitivity of the method. In short, early wetting of the electrode membrane in water-diluted cutting fluids and oils occurred, preventing analysis. Methods were chosen in order to avoid early wetting. With cutting fluids, large dilutions were made and, with cutting oils, solvent extraction was used partially to break the emulsion. These methods will be discussed further in the following sections.

The third type of interference (*e.g.*, weak acids) was also encountered. The effect of the presence of this type of interference was to cause the electrode to respond as if nitrite ion were present. The selectivity constants of some weak acids have been determined[26] and their effects during this work were avoided by a standard-subtraction method discussed in the next section.

Because iodide salts are included in the general formulations of cutting fluids,[3] the effect of 0.1 M potassium iodide solution was investigated and found to be a weak but positive interference. The selectivity constant was determined to be about 0.012. The effect of an iodine solution was similar.

For the same reason, mono-, di- and triethanolamine were investigated in the same manner and also by adding small amounts to a 0.46 μg ml^{-1} nitrite ion standard solution during analysis. No interferences were observed during 15 min.

Several other salts were investigated to identify a suitable salting-out reagent to be used in a solvent-extraction emulsion-breaking step. Saturated calcium sulphate (about 0.02 M), 0.1 M potassium dichromate and 0.1 M sodium sulphate solutions were found not to interfere in the analysis of 0.046 and 0.46 μg ml^{-1} nitrite ion solutions. However, 0.1 M sodium chloride solution did interfere positively. This agrees only partially with Tabatabai,[32] who reported that 0.1 M sodium chloride solution did not interfere but a 1 M solution did.

Calibration of the Electrode

Because the method of standard additions has been recommended for use with electro-analytical techniques by several workers,[33,34] it was considered for use during this work. While this method can, for any technique, compensate for enhancement or reduction in the response of the analyte brought about by any unmatched part of the matrix (direct comparison with similar standards cannot do this), it cannot in any technique compensate for an

interfering species that causes a response like that of the analyte or one that removes the analyte before measurement.[35] Hence, during this work standard additions could not compensate for the presence of any of the above interfering weak acids. An additional complication arising when the method of standard additions is applied to potentiometric measurements is that compensation for enhancement of the analyte response due to the unmatched part of the sample matrix can only be accomplished by using an iterative computer program.[27,36–39]

In order to avoid interfering weak acids, present during this work, a standard-subtraction method was adopted. The agent used to remove nitrous acid was sulphamic acid, chosen because the reaction was reported to be selective, rapid and quantitative.[25]

Interfering species of the other type, *i.e.*, those which cause enhancement or suppression of the response of the analyte, were investigated by analysing several spiked nitrite-free cutting oils. No significant effects were observed. This was a necessary criterion in order to ensure accuracy for this standard-subtraction method.

The addition of sulphamic acid did not seem to have any serious effect on the electrode performance. However, the inner pH electrode was raised and lowered between each analysis and then equilibrated in a standard sodium nitrite solution to minimise the memory effects of species that could have moved across the membrane.

Sulphamic acid additions had no effect on the electrode potential when only the following positive interfering species, listed in order of increasing K_x values, were analysed: potassium iodide, sodium chloride, acetic acid, hydrofluoric acid, formic acid, lactic acid and pyruvic acid.

Analysis of Cutting Fluids and Cutting Oils

To our knowledge only three methods of nitrite detection have been applied to cutting fluids and cutting oils, but none has been demonstrated to be useful at low microgram per millilitre levels, a requirement with which this laboratory was charged. Merckoquant nitrite test papers have been employed,[40] which in our experience turned various shades of pink after being dipped briefly into standard sodium nitrite solutions of 1 μg ml^{-1} of nitrite ion or higher. However, their limit of detection was raised considerably when applied directly to coloured cutting fluids or oils. In our opinion, they could safely be used for lubricants containing 30 μg g^{-1} of nitrite ion or higher. The evolution of brown fumes (nitrogen dioxide) after addition of a strong acid has also been employed[41] and this could have been the basis of a sensitive and selective method if a suitable technique for measuring nitrogen dioxide, such as headspace-gas chromatographic analysis, was used. Lastly, Williams *et al.*[9] applied a standard spectrophotometric procedure for water and waste water samples[20] to several high-nitrite cutting fluids collected in the Ottawa region.

Cutting fluids

We were fortunate to obtain the same samples that were analysed by Williams *et al.* for our use. Our analyses of them (Table II) confirmed the presence of high levels of nitrite salts in these cutting fluids. Slightly lower concentrations were found in two out of six additional cutting fluids tested. It was not surprising to find four of our results comparatively lower than those of Williams *et al.*, as triethanolamine is also present in these cutting fluids[9] and this compound can react slowly over a period of months[6,9,10] with nitrite ion in the product itself.

While direct analysis of the cutting fluids was made after a large dilution with distilled water (to about 300 *V*/*m*) because of the high nitrite ion concentrations, smaller dilutions and hence lower detection limits might be possible. However, an optimum dilution factor existed for these samples as membrane wetting took place quickly when immersion into a fluid diluted by the factor of 10 *V*/*m* was made. Assuming a dilution factor of 300 *V*/*m* and a lower limit of aqueous nitrite detection of 0.046 μg ml^{-1}, the lower detection limit for nitrite ion in cutting fluids was 17 μg g^{-1}.

During the analysis of these cutting fluids it was demonstrated that the standard-subtraction step was necessary to avoid erroneously high results (Table II) in six instances.

The Merckoquant test papers gave a positive indication of nitrite ion presence in fluids 1–8 and negative indication in fluids 9–12.

About 20 cutting fluids could be analysed in 8 h.

TABLE II

CONCENTRATION OF NO_2^- ION FOUND IN DIFFERENT CUTTING FLUIDS

Cutting fluid	NO_2^- concentration/$\mu g\ g^{-1}$		
	This work		
	SS* used	SS* not used	Williams *et al.*[9]
1	6.0×10^4	—	8.2×10^4
2	2.9×10^4	—	3.4×10^4
3	9.0×10^4	—	9.8×10^4
4	2.0×10^3	—	2.0×10^3
5	1.3×10^5	—	8.6×10^4
6	3.1×10^4	—	3.8×10^4
7	1.8×10^2	1.9×10^3	—
8	2.3×10^2	2.0×10^3	—
9	<14	6.5×10^2	—
10	<14	85	—
11	<16	1.5×10^3	—
12	<16	1.4×10^3	—

* SS = Standard-subtraction method.

Cutting oils

Simple dilution and measurement of cutting oils proved unsuccessful during this work. Wetting of the membrane took place within 60 s of immersion in a cutting oil with a dilution factor of 1000 *V/m*. As a result, a solvent-extraction step was developed in order to separate the nitrite ion from the wetting agents. Although more efficient separations could have possibly been used, this separation fulfilled the sensitivity requirement placed on this laboratory.

After determining that cleaner aqueous phases were obtained when the cutting oil was dissolved in the solvent phase first, rather than in the aqueous phase, the utility of several organic solvents was investigated by agitating 100 ml of 4.6 $\mu g\ ml^{-1}$ standard nitrite ion solution with 100 ml of organic solvent and then analysing the aqueous phase. *p*-Xylene (Eastman 277), hexane mixture (Fisher H-292) and *s*-tetrachloroethane (Fisher A-31) gave, for undetermined reasons, recoveries of only 10% or less. Of the suitable solvents practical-grade 2,2,4-trimethylpentane (TMP) was used because it was inexpensive and available in large amounts.

A restriction on the sample size of the cutting oil was observed to limit the sensitivity of the method just as with cutting fluids. Using 100-ml volumes of TMP and aqueous phases, aliquots of cutting oil of 1 g or greater caused the electrode potential to be erratic and the membrane life to be greatly reduced. The optimum aliquot seemed to be between 0.2 and 0.4 g, but even then the membrane required changing after about 45 min of immersion. With some oils or with smaller aliquots longer immersion times were possible.

The efficiency of the solvent-extraction step was investigated by analysing, in quadruplicate, a nitrite-free cutting oil, which was spiked so that after extraction the aqueous phase contained 2.3 $\mu g\ ml^{-1}$ of nitrite ion. As aqueous phases, 0.05 M sodium sulphate and 0.01 M potassium dichromate solutions were found to be unsuitable because they allowed recoveries of only 89% and 72% of nitrite ion, respectively, whereas distilled water and calcium sulphate solution allowed about 100% recoveries (Table III). Saturated calcium sulphate solution is recommended for use as it produced, according to the electrode performance, slightly cleaner aqueous phases possibly owing to its emulsion-breaking properties.

To investigate the recovery at a much lower nitrite ion concentration, *i.e.*, 0.046 $\mu g\ ml^{-1}$ in the aqueous phase, another spiked nitrite-free cutting oil was analysed in quadruplicate, using saturated calcium sulphate solution as the aqueous phase. The average recovery was 100% but a larger relative standard deviation was observed (Table IV).

The solvent-extraction step was also shown to be suitable for cutting fluids if the need, however unlikely, ever arose (Table V). All three aforementioned aqueous phases were used to analyse, in quadruplicate, cutting fluid 8. The results were not statistically different from each other (using analysis of variance test with a 95% confidence limit).

TABLE III

CONCENTRATION OF NO_2^- FOUND IN AQUEOUS PHASES AFTER EXTRACTION OF A NITRITE-FREE CUTTING OIL, SPIKED SO THAT THE AQUEOUS PHASES CONTAINED 2.3 μg ml^{-1} OF NO_2^- ION

	NO_2^- concentration in aqueous phase*/μg ml^{-1}			
	DW	A	B	C
	2.14	2.04	1.96	1.72
	2.28	2.25	2.14	1.79
	2.28	2.37	2.04	1.61
	2.41	2.56	2.08	1.46
Mean	2.28	2.31	2.05	1.65
Standard deviation	0.11	0.22	0.08	0.14
Recovery, %	99	100	89	72

* DW = distilled water; A = saturated $CaSO_4$; B = 0.05 M Na_2SO_4; C = 0.01 M $K_2Cr_2O_7$.

Of the 20 cutting oils analysed only one was found to contain a detectable nitrite ion concentration (16 μg g^{-1}). The other oils, by definition, contained less than about 15 μg g^{-1} of nitrite ion. Also, in only two instances (neither of the above) was the standard-subtraction step necessary to avoid erroneous results equivalent to 150 and 41 μg g^{-1} of nitrite ion.

TABLE IV

CONCENTRATION OF NO_2^- ION FOUND IN A SATURATED $CaSO_4$ AQUEOUS PHASE AFTER EXTRACTION OF A NITRITE-FREE CUTTING OIL SPIKED SO THAT THE AQUEOUS PHASE CONTAINED 0.046 μg ml^{-1} OF NO_2^- ION

	Cutting oil	NO_2^- concentration in aqueous phase/μg ml^{-1}	Recovery, %
	32	0.041	89
	24	0.044	96
	23	0.052	113
	25	0.046	100
Mean	..	0.046	100
Standard deviation	..	0.005	10

The Merckoquant test papers did not indicate the presence of nitrite ion in any of the cutting oils.

About 10 cutting oils could be analysed during 8 h.

TABLE V

CONCENTRATION OF NO_2^- ION FOUND IN CUTTING FLUID 8 USING DIFFERENT AQUEOUS PHASES

	NO_2^- concentration in aqueous phase*/μg g^{-1}				
	DW	A	B	C	WD
	215	203	231	220	238
	235	216	244	234	246
	238	211	238	230	247
	227	213	245	236	245
Mean†	229	211	240	231	244
Standard deviation† ..	10	6	6	8	4

* As in Table III; WD = water-diluted sample.
† Over-all mean = 231 μg g^{-1}; over-all standard deviation = 13 μg g^{-1}.

Used cutting fluids and oils

No attempt was made to apply these methods to used cutting fluids or used cutting oils. To do so would bring up the following two considerations, depending on the particular use of the lubricant.

The most important consideration would be that the used lubricant would then be an oil in water emulsion that would have to be broken in such a way as to release the nitrite ion quantitatively to aqueous solution. As this could not be done efficiently enough for our needs (stated previously) by agitating the emulsion with TMP or other solvents, another emulsion-breaking step would have to be used. Examples of such techniques are the addition of ethanol, the addition of salts, boiling, freezing and vigorous agitation. We feel that the last two would be the most promising.

The second consideration would be that additional contaminants would be added to the emulsion during use of the lubricant. Three main contaminants could be wear metals, surfactants (possibly used to clean the emulsion off the worked material) and tap water (used to make the emulsion). Only the presence of surfactants (wetting agents) would prevent the optimum use of this method but they should also be separated from nitrite ion with the proper choice of emulsion-breaking step.

Conclusion

We have found the methods of nitrite ion determination described herein to meet our needs for rapidity, economy and sensitivity. The detection limits for cutting fluids and oils were about 15 $\mu g\ g^{-1}$. In addition, positive interference from weak acids could be avoided by a standard-subtraction method using sulphamic acid. The major drawbacks of the methods were the restrictions placed on the sensitivities due to the required sample dilutions and the extra time required for membrane replacement.

We thank David T. Williams (Environmental Health Directorate, Health Protection Branch, Ottawa, Canada) for providing us with several previously analysed cutting fluids, the several lubricant manufacturing companies who provided us with numerous other lubricants and John A. Page (Queen's University, Kingston, Canada) for helpful discussions.

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Received *August* 10*th*, 1978
Accepted *October* 17*th*, 1978

SHORT PAPERS

Dicarboxidine [γ,γ′-(4,4′-Diamino-3,3′-biphenylylenedioxy)dibutyric Acid] as a Reagent for the Spectrophotometric Determination of Cyanide and Chlorine

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Keywords: Dicarboxidine [γ,γ′-(4,4′-diamino-3,3′-biphenylylenedioxy)dibutyric acid] chromogen; cyanide determination; chlorine determination; spectrophotometry

Benzidine, *o*-tolidine and *o*-dianisidine are reagents with wide applications, *e.g.*, in the detection of blood,[1,2] and the spectrophotometric determination of cyanide,[3] chlorine,[4] compounds containing the –CO–NH– group[5,6] and manganese.[7] However, the carcinogenic properties of these chromogens necessitate the search for safer alternatives.

Dicarboxidine [γ,γ′-(4,4′-diamino-3,3′-biphenylylenedioxy)dibutyric acid] is described by Jönsson *et al.*[8] as being significantly less carcinogenic than benzidine and its derivatives. Svahn and Gyllander[9] have been able to replace *o*-tolidine and *o*-dianisidine with dicarboxidine in the detection of *N*-protected amino acids, peptides, barbiturates and meprobamate on thin-layer chromatograms.

This paper describes the use of dicarboxidine as a reagent for the spectrophotometric determination of cyanide and chlorine. Optimum reaction conditions are evaluated and the sensitivity of the method is compared with those using benzidine and *o*-tolidine as the chromogen.[3,4] The reaction with cyanide permits the determination of cyanocobalamin in tablets after liberation of hydrogen cyanide.

Experimental

Apparatus

A Zeiss PMQ II spectrophotometer with 1-cm cuvettes was used. The absorption spectra were recorded on a Shimadzu, Model MPS-5000, Multipurpose Recording Spectrophotometer.

Reagents

All chemicals were of analytical-reagent grade and were used without additional purification. The purity of the dicarboxidine dihydrochloride was verified by high-voltage paper electrophoresis at pH 3.6.

For the determination of cyanide

Orthophosphoric acid, 0.04 M.

Bromine solution, not necessarily saturated.

Sodium arsenite solution, 2% *m/V.*

Butan-2-ol.

Ethanol, 95%.

Pyridine solution. Dissolve 125 ml of pyridine in 375 ml of water and add 10 ml of concentrated hydrochloric acid.

Dicarboxidine solution. Dissolve 0.3 g of dicarboxidine dihydrochloride in 25 ml of water and filter if necessary.

Pyridine - dicarboxidine reagent. Immediately before use, dilute 5.00 ml of dicarboxidine solution to 50.0 ml with pyridine solution.

Standard cyanide solutions. Prepare a stock solution containing 150 mg l^{-1} of potassium cyanide and dilute aliquots as required.

For the determination of chlorine

Dicarboxidine reagent. Dissolve 3.3 g of dicarboxidine dihydrochloride in 1000 ml of 3×10^{-3} M hydrochloric acid; this solution is stable for several days.

Standard chlorine solutions. Prepare a stock solution of chlorine gas in water, standardise this solution by iodimetric titration with thiosulphate immediately before use and dilute aliquots with water as required.

Procedures

Determination of cyanide

Mix 2.00 ml of 0.04 M orthophosphoric acid with 2.00 ml of sample (containing 0.4–2 mg l^{-1} of cyanide in water) in a centrifuge tube. Add 1 drop of bromine water and 2 drops of sodium arsenite solution followed by 6.00 ml of butan-2-ol. Shake the mixture, add 2.00 ml of pyridine - dicarboxidine reagent, then shake the mixture vigorously for 2 min and allow it to stand at room temperature for 15 min. Centrifuge, then mix 4.00 ml of the organic layer with 1.00 ml of ethanol and measure the absorbance of this solution at 490 nm against a reagent blank prepared from distilled water. Prepare a standard graph from the standard cyanide solutions.

Determination of chlorine

To 50.00 ml of sample (containing 0.5–3 mg l^{-1} of chlorine) add 2.50 ml of 3 M nitric acid and 2.50 ml of dicarboxidine reagent. Allow the solution to stand at room temperature for 30 min and measure the absorbance at 460 nm against a reagent blank prepared from chlorine-free water. Prepare a standard graph from the standard chlorine solutions.

Results and Discussion

Determination of Cyanide

The yellowish reaction product between cyanide and pyridine - benzidine has an absorbance maximum at 480 nm[3] whereas pyridine - dicarboxidine gives a red product with an absorbance maximum at 490 nm. The stability and development time of the two colours are similar.

The colour with pyridine - dicarboxidine reaches maximum intensity almost immediately after addition of the pyridine - dicarboxidine reagent and fades slowly with time. Thirty minutes after mixing the sample and colour reagent, the absorbance decreases to 96.5% of

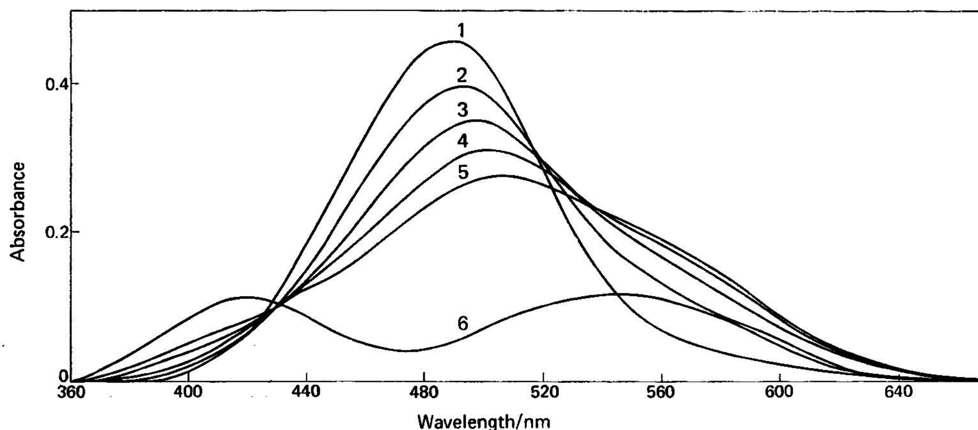

Fig. 1. Stability of the colour developed from cyanide and pyridine - dicarboxidine. Sample solution: 0.84 mg l^{-1} of cyanide treated according to the described method. Spectra: 1, immediately after mixing; 2, 1.5 h after mixing; 3, 3 h after mixing; 4, 4.5 h after mixing; 5, 6 h after mixing; 6, 22 h after mixing.

the value read after the 15-min period proposed in the method. Fig. 1 illustrates the stability of the colour, and as can be seen the absorbance maximum is shifted with time to longer wavelengths.

The calibration graph is rectilinear in the concentration range 0.2–2.7 mg l^{-1} of cyanide (in the working solution). The sensitivity is about 70% of that given by the pyridine - benzidine reagent.

The concentration of dicarboxidine dihydrochloride in the reagent appears not to be critical, as concentrations between 1.3×10^{-3} and 2.6×10^{-3} mol l^{-1} of dicarboxidine in the pyridine - dicarboxidine reagent gave identical absorbance values.

The method is used in our laboratory for the routine determination of cyanocobalamin in tablets. Hydrogen cyanide is first quantitatively liberated by reduction with tin(II) chloride according to the procedure of Dessouky and Pungor.[10] Amounts of 50 μg of cyanocobalamin are used in the determination and the method has a relative standard deviation of about 2%.

Determination of chlorine

In the determination of free chlorine using the *o*-tolidine method, sodium bis(2-ethylhexyl)sulphosuccinate is added in order to stabilise the blue quinhydroneimino product formed. When this stabilised neutral *o*-tolidine procedure[4] was applied using dicarboxidine dihydrochloride (7×10^{-3} mol l^{-1}) instead of *o*-tolidine, a yellow colour with an absorbance maximum at 438 nm was obtained, which, however, was too faint to be used in practical measurements.

Some modifications of the method, omitting the sodium bis(2-ethylhexyl)sulphosuccinate stabiliser,[4] were therefore tried. By simply mixing the sample solution, reagent and phosphate buffer (pH 7.4), a greenish colour was obtained with an absorbance maximum at about 460 nm. The absorbance, however, was low and the standard graph was not rectilinear below 0.1 mg l^{-1} of chlorine. Changing the pH from 7.4 to 2 resulted in an unstable, intensely red colour with an absorbance maximum at 460 nm.

By further lowering the pH to about 1 with nitric acid, as indicated under Procedure, a more stable red colour with an absorbance maximum at 460 nm was obtained. A spectrum recorded immediately after mixing a sample containing 3 mg l^{-1} of chlorine with nitric acid and dicarboxidine reagent is presented in Fig. 2. The absorbance decreases with time, but for more dilute chlorine solutions (0.5–1 mg l^{-1}) the absorbance increases with time. This is illustrated in Fig. 3, which shows standard graphs obtained 2, 35 and 45 min after addition of acid and dicarboxidine reagent. After 35 min, the graph is rectilinear and passes through the origin, while deviations from the origin and over-all lower absorbance values are obtained after 45 min. The absorbance maximum does not change during the time studied. At a

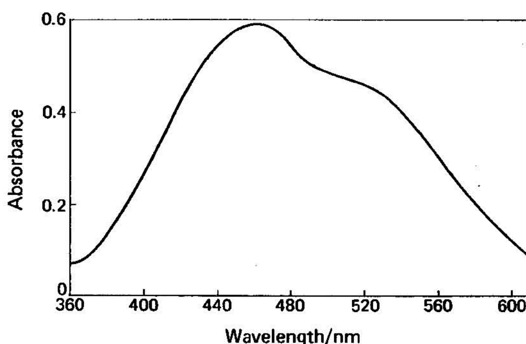

Fig. 2. Spectrum of the dicarboxidine product obtained from the reaction with chlorine. Sample solution: 2.95 mg l^{-1} of chlorine treated according to the proposed method at pH 1. The spectrum was recorded within a few minutes after the addition of dicarboxidine reagent.

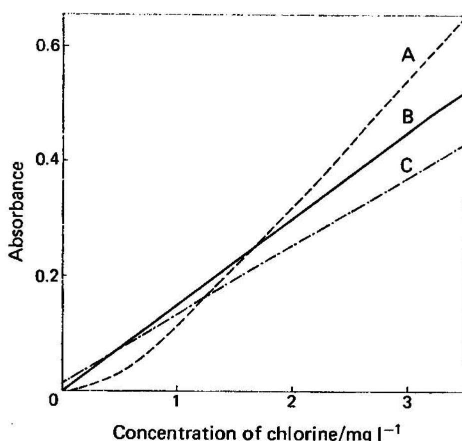

Fig. 3. Standard graphs for chlorine determined with dicarboxidine. Time after addition of dicarboxidine reagent: A, 2; B, 35; and C, 45 min.

concentration level of 3 mg l^{-1} of chlorine the absorbance after 35 min had decreased to 82% and after 45 min to 69% of the value obtained after 2 min. For practical measurements it would be preferable to wait 30 min before measuring the absorbance.

The dicarboxidine method described above appears to give about the same sensitivity as *o*-tolidine used as described in the literature.[4] The reason for the different behaviour at lower and higher chlorine concentrations has not yet been investigated and needs additional studies. Although the method is useful in its present form, it is obvious that a suitable stabiliser for the colour would be advantageous. No attempts have hitherto been made to effect this improvement.

The author thanks Mr. Lars Jansson for skilful technical assistance and Dr. N. Å. Jönsson for valuable discussions of the manuscript.

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Received *October* 19*th*, 1978
Accepted *November* 7*th*, 1978

Semi-automatic Determination of Manganese in Natural Waters and Plant Digests by Flow Injection Analysis

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Keywords: Manganese determination; water analysis; plant material analysis; flow injection analysis; spectrophotometry

The formaldoxime method[1] has been applied extensively to the spectrophotometric determination of manganese, because of its high sensitivity and the rapid formation of a stable coloured complex. However, iron also forms a strongly coloured complex with formaldoxime and this causes serious interference.

Formaldoxime ($H_2C{=}NOH$) can be obtained in a solution acidified with hydrochloric acid by reaction of hydroxylammonium chloride with formaldehyde. Formaldoxime is dissociated in an alkaline medium, producing anions ($H_2C{=}NO^-$) that form coloured complexes with multivalent metals (manganese, iron, copper, cobalt, nickel, cerium and vanadium).[2]

The physical and chemical conditions of this reaction permit the use of the continuous-flow injection system developed by Růžička and co-workers.[3–5] This technique utilises the introduction of the sample into a continuously flowing carrier stream of water or reagent. When injected, the sample is pushed by this stream and dispersed into the reagent stream, whereupon the required reaction takes place. The coloured complex is then carried into a spectrophotometric flow cell, where the absorbance is measured after an exactly defined time interval.

Experimental

Reagents

Formaldoxime solution, 1 M. Dissolve 7 g of hydroxylammonium chloride in water, add 3 ml of formaldehyde solution (37% *m/V*) and dilute to 100 ml with water. This solution must be prepared weekly and stored in a glass bottle.

Ascorbic acid solution, 5% *m/V*.

Potassium cyanide solution, 10% *m/V*.

Methyl orange solution, 0.002% *m/V in phosphate buffer*, pH 7.0.

Phosphate buffer. Dissolve 0.512 g of potassium dihydrogen orthophosphate and 1.034 g of dipotassium hydrogen orthophosphate in water and dilute to 1 l with water. This solution is 0.01 M in phosphate, pH 7.0.

Sodium hydroxide solution, 6 M.

Hydrochloric acid, 0.4 M.

Standards

Standard manganese solution, 100 p.p.m. Dissolve 0.308 g of manganese(II) sulphate ($MnSO_4.H_2O$) in about 800 ml of water, add 13 ml of concentrated nitric acid and dilute to 1 l with water.

Working standard solutions. Working solutions containing manganese in the range 0.05–5 p.p.m. are prepared weekly by appropriate dilution of the stock solution.

Apparatus and Procedure

The system employed for this work is shown in Fig. 1. Polyethylene tubing of 0.86 mm i.d. was used and all connectors were made from Perspex. The pump was a Technicon AAII peristaltic pump, fitted with Tygon pumping tubes.

The samples were injected by means of a proportional injector.[6] The sample was aspirated to fill a loop, which exactly defined the injected volume; this loop was then placed as part

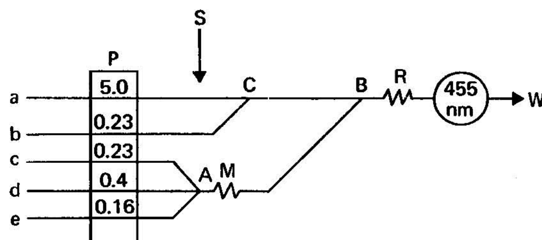

Fig. 1. Flow diagram of the system. P, peristaltic pump; S, injection port; R, reaction coil (length 30 cm); M, mixing coil (length 60 cm); and W, waste. The numbers in the pump are the flow-rates in millilitres per minute of the carrier, reduction, reagent, neutralisation and masking streams, which correspond to (a), (b), (c), (d) and (e), respectively. The distances S–C and C–B are approximately 2 cm. For details, see text.

of the carrier stream. Water samples were injected without any pre-treatment, other than preservation (5 ml of 2 M sulphuric acid were added per 1000 ml of sample, immediately after collection) and plant samples were digested with nitric - perchloric acid using a Technicon BD-40 block digestor.[7]

Formaldoxime, masking and neutralisation streams are added to each other at point A (Fig. 1) and mixed by passage through coil M. At point B, the reagents meet the sample zone, which had previously received a reduction stream of ascorbic acid at point C. The formation of the coloured complex takes place in the reaction coil (R) and the absorbance is measured at 455 nm in a Beckman, Model 25, spectrophotometer, connected to a Beckman, Model 24-25 ACC, recorder, and equipped with a Hellma Type 178 flow cell, light path 10 mm, volume 0.08 ml.

For the analysis of waters, the carrier stream was water, while for the analysis of plant digests, the acidic conditions of the samples were maintained in the carrier stream. Consequently, the sodium hydroxide concentration in the neutralisation stream was changed, in order to achieve the required alkaline conditions in the final stream.

In order to prevent the reaction between iron(II) and formaldoxime, another iron complexing agent (potassium cyanide) was used, in the presence of a reductant (ascorbic acid) that reduces iron(III) to iron(II). Aluminium, titanium, uranium, molybdenum and chromium also form light-coloured complexes that normally do not interfere in the determination of manganese in water or plant material by this method. If the aluminium or titanium concentrations are higher than 40 p.p.m. an additional masking flow of tartrate is recommended.[2]

The effect of the presence of suspended and coloured materials in the sample was evaluated by replacing the formaldoxime with water and running the samples again, so as to obtain blank values.

Results and Discussion

The proposed system (Fig. 1) was designed in order to optimise the sampling rate, reagent composition and consumption of sample and reagent. The carrier stream was chosen to have a flow-rate of 5 ml min^{-1}, in order to obtain a desirable speed of analysis with a satisfactory degree of sample dispersion, as will be discussed later. The other flow-rates were fixed as specified in Fig. 1. Under these conditions, the reagent streams have little influence on the sampling rate and dilute the carrier stream (or sample zone) by a theoretical factor of only 0.86, which was confirmed by tests using a methyl orange solution.

The composition of the reagents was established as follows.

Carrier stream. In order to avoid loss of sensitivity caused by differences between the refractive index of sample and carrier stream,[8] the latter should be water for the analysis of water samples and 0.4 M hydrochloric acid for plant samples.

Neutralisation stream. Incomplete neutralisation results in a distorted peak. In order

TABLE I

INFLUENCE OF INJECTED VOLUME ON SENSITIVITY AND SAMPLING RATE

Standard solution contained 2 p.p.m. of manganese.

Volume injected/ ml	Absorbance	Relative sensitivity, %*	Sampling rate/ samples per hour†
0.0580	0.115	16.3	230
0.1611	0.189	25.8	167
0.2323	0.309	42.9	143
0.3485	0.399	54.1	136
0.4647	0.468	64.7	120
∞‡	0.637	88.0	—

* Data obtained with methyl orange solution.[5]
† Data obtained after examination of the shape of the peak in Fig. 2.[9]
‡ "Infinite volume" represents the conditions when the carrier stream was replaced by sample.

to maintain an adequate alkalinity (pH greater than 9.0), 6.0 or 1.0 M sodium hydroxide solution was used as the neutralisation stream for the analysis of plant extracts or water samples, respectively.

Masking stream. The interference of iron was minimised by using ascorbic acid and potassium cyanide in the amounts specified above. In this situation, the iron(III) interference was suppressed when its concentration was below 20 p.p.m. In practice, the concentration of this interferent in nitric - perchloric acid digests of plants is less than 15 p.p.m. and seldom reaches 1 p.p.m. in natural waters. In special instances of samples with higher contents of iron(III) the concentration of masking reagent must be increased.

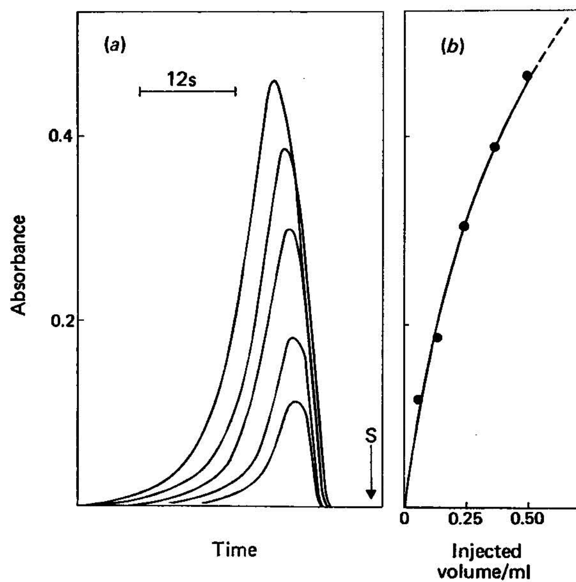

Fig. 2. (a), Peaks corresponding to different injected volumes of a 2 p.p.m. manganese standard. S is the injection point. (b), Shows the influence of the injected volume on the peak height. For details, see Table I.

Formaldoxime solution. The effect of formaldoxime concentration on the slope and linearity of calibration graphs was studied by varying the reagent concentration (0.5, 1.0, 1.5 and 2.0 M). Linear calibration graphs from 0.1 to 2.0 p.p.m. of manganese were obtained

by using formaldoxime solutions in the concentration range between 1.0 and 2.0 M. Higher blank values (probably due to refractive index differences)[8] were detected when the formaldoxime concentration was increased and loss of sensitivity resulted when more diluted formaldoxime solutions were used. As a compromise, a 1.0 M formaldoxime solution was chosen as standard.

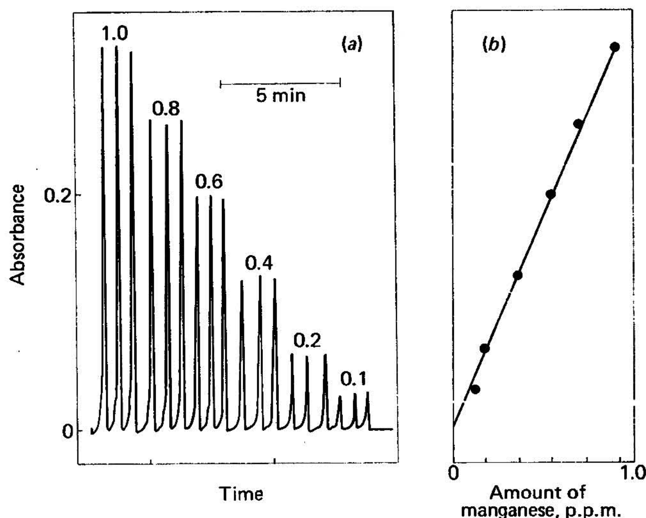

Fig. 3. (*a*), Calibration peaks. The values on the peaks represent p.p.m. of manganese. Chart speed: 5 min in^{-1}. (*b*), Corresponding calibration graph.

The dispersion of the sample, which is related to the volume injected, is a critical parameter that influences the sensitivity and sampling rate.[5] The results presented in Table I and Fig. 2 were obtained with the system shown in Fig. 1 and indicate the dependence of the sensitivity and sampling rate on the injected volume, which was changed by variation of the sample loop in the proportional injector. The relative sensitivity is the ratio between the dispersion of the actual injected volume and that of an infinite volume and was determined by using a methyl orange solution according to the procedure described by Růžička and Hansen.[5] The sampling rate was evaluated after examination of the recorded peak

TABLE II

COMPARISON OF RESULTS OBTAINED BY CONTINUOUS FLOW INJECTION AND ATOMIC-ABSORPTION SPECTROPHOTOMETRY

Concentration of manganese, p.p.m.			
Water samples		Plant digests	
Flow injection	AAS*	Flow injection	AAS*
0.08	0.07	1.63	1.58
0.20	0.22	1.58	1.53
0.28	0.30	0.50	0.55
0.08	0.09	0.46	0.53
0.07	0.06	0.70	0.85
0.12	0.12	0.50	0.53
0.07	0.07	1.61	1.63

* Atomic-absorption spectrophotometry.

shape (Fig. 2) as recommended by Růžička and Stewart.[9] The good correlation between the results in columns 2 and 3 (Table I) indicates that completion of the chemical reactions is achieved.

A series of determinations were performed using the system shown in Fig. 1, with an injected volume of 0.35 ml. Under these conditions, about 135 samples per hour could be analysed, with a standard deviation of better than 1% over the range 0.2–2 p.p.m. of manganese. Fig. 3 shows a typical calibration run and the corresponding calibration graph.

In order to check the accuracy of the proposed method, a comparison of this method with atomic-absorption spectrophotometry[10] was made. Some individual differences were found and no attempt was made to study the problem. The results are presented in Table II and indicate no statistical difference at the 1% level, for both plant material and water analysis.

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Received *August 1st*, 1978
Accepted *September 27th*, 1978

Gravimetric Determination of Copper(II) and Cobalt(II) by Selective Precipitation with Benzimidazole

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Keywords: *Copper determination*; *cobalt determination*; *benzimidazole*; *gravimetry*

Fiegl and Gleich[1] investigated the formation of salts with benzimidazole by some metal ions, such as mercury(II), copper(II), cadmium, zinc and cobalt(II). According to these workers the copper salt is precipitated from an ammoniacal solution of a copper salt and the cobalt salt is precipitated from an ammoniacal solution in the presence of ammonium chloride. The violet - blue crystalline precipitate obtained in the presence of cobalt has been used for its detection; nickel does not interfere as the nickel salt is not precipitated immediately under similar conditions, but on standing for over 12 h a grey - violet precipitate forms.[2] Yogada[3] has used the reaction between cobalt ions and benzimidazole as the basis of a semi-quantitative method for the determination of cobalt by a spot test. Ghosh and Ghosh[4] reported the formation of a stable cobalt(II) complex with benzimidazole and on that basis determined cobalt gravimetrically. According to these workers the precipitation is carried out at pH 10 and therefore all elements forming insoluble hydroxides must be absent.

From a review of the literature it appears that although benzimidazole forms insoluble precipitates with several metal ions, its role as a quantitative precipitant has not been studied systematically. The present work was therefore undertaken with a view to determining copper(II) and cobalt(II) separately and in a single solution by the use of benzimidazole as a quantitative precipitant under controlled pH conditions. Determinations of copper and cobalt were carried out separately and then in solutions containing both ions. Interferences by Ni^{2+}, Cd^{2+}, Zn^{2+}, Bi^{3+}, Sb^{3+} and Be^{2+} were examined. Nickel did not interfere but serious interferences were observed with the other ions.

Experimental

Reagents

All chemicals used were of analytical-reagent grade. Benzimidazole was prepared and purified by the method described in the literature.[5] A stock solution (0.04 M) of the reagent was prepared in doubly distilled water and was made slightly acidic with hydrochloric acid. The solutions of copper(II) and cobalt(II) were prepared from their sulphate salts and were standardised before use.

Apparatus

pH measurements were made using a Metrohm Herison pH meter, Model E 520, and electrodes.

Methods

Determination of copper in a solution containing only copper(II) ions

Place 5–20 ml of the copper solution (5–20 mg of Cu) in a 400-ml beaker, add 10–40 ml of the reagent solution, dilute to about 150 ml and heat the solution almost to boiling. Raise the pH of the solution slowly by adding dilute ammonia solution, drop by drop, with constant stirring until an additional drop does not form a precipitate. It was found that precipitation started at pH 4.8 and was quantitative at pH 5.5. Heat the mixture on a hot water-bath for 15 min, then filter through a weighed sintered-glass crucible, porosity G4, wash the precipitate with 0.1% reagent solution, then three times with distilled water and finally with 50% ethanol. Dry at 110 °C for 2 h and weigh (Table I). The conversion factor for $Cu(C_7H_5N_2)_2$ to copper is 0.21339.

Determination of cobalt in a solution containing only cobalt(II) ions

Place 5–20 ml of the cobalt solution (5–20 mg of Co) in a 400-ml beaker, add 10–40 ml of the reagent solution, dilute to about 150 ml and heat the solution almost to boiling. Raise the pH of the solution by adding ammonia solution, drop by drop, with constant stirring until an additional drop of ammonia solution does not form a precipitate. The precipitation in this instance starts at pH 7 and is quantitative at pH 8.2. After the precipitation is complete follow the same procedure as outlined for copper (Table I). The conversion factor for $Co(C_7H_5N_2)_2$ to cobalt is 0.2010.

Determination of copper and cobalt when present in the same solution

To a solution containing a mixture of copper(II) and cobalt(II) ions, add an amount of the reagent solution nearly double that necessary for complete precipitation of the metal ions, dilute the solution and heat it almost to boiling. Add ammonia solution, drop by drop, with constant stirring until the pH of the solution reaches 5.5. Heat the solution on a water-bath, filter, wash, dry and weigh the precipitate as described above for copper. Collect the filtrate and washings, boil the solution and add a further amount of ammonia solution dropwise with constant stirring until the solution attains a pH of 8.2. Heat, filter, wash, dry and weigh the precipitate as described for cobalt (Table II).

NOTE—

The pH values are critical for the precipitation of both copper and cobalt. The freshly precipitated copper compound tends to dissolve in ammonia solution above pH 7 and the cobalt compound above pH 10.

TABLE I

GRAVIMETRIC DETERMINATION OF COPPER(II) AND COBALT(II) IN SEPARATE SOLUTIONS USING BENZIMIDAZOLE AS PRECIPITANT

All results reported are the means of three determinations.

Mass of Cu taken/ mg	Mass of precipitate/ mg	Calculated mass of $Cu(C_7H_5N_2)_2$/ mg	Error, %	Mass of Co taken/mg	Mass of precipitate/ mg	Calculated mass of $Co(C_7H_5N_2)_2$/ mg	Error, %
5	23.3	23.415	−0.45	5	24.8	24.850	−0.2
10	47.0	46.830	+0.36	10	49.9	49.700	+0.40
15	70.5	70.245	+0.36	15	74.3	74.550	−0.34
20	93.9	93.660	+0.25	20	99.8	99.400	+0.40

Results and Discussion

As is evident from the results in Tables I and II, benzimidazole can be used as an effective quantitative precipitant for copper(II) and cobalt(II) ions. By careful control of the pH the determination of these two ions can be carried out in a single solution. The fact that nickel does not interfere is an added advantage of the method because nickel is found together with these metals in many materials. The pH ranges are selective for both copper and cobalt. Whereas the freshly precipitated copper compound tends to dissolve in ammonia solution above pH 7, that of cobalt is not affected up to pH 10. The results of the analysis are broadly in agreement with the compositions of the complexes reported for these metals earlier.[1] The colour of the copper complex was chocolate brown and that of the cobalt blue - violet. The compounds were found to be stable and no loss of mass was noticed when either compound was heated to 300 °C.

TABLE II

GRAVIMETRIC DETERMINATION OF COPPER(II) AND COBALT(II) IN A SINGLE SOLUTION USING BENZIMIDAZOLE AS PRECIPITANT

All results reported are the means of three determinations.

Mass of Cu taken/ mg	Mass of Co taken/ mg	Approximate molar ratio of Cu to Co	Mass of copper precipitate (pH 4.8–5.5)/ mg	Calculated mass of $Cu(C_7H_5N_2)_2$/ mg	Mass of cobalt precipitate (pH 7–8.2)/ mg	Calculated mass of $Co(C_7H_5N_2)_2$/ mg	Error, %	
							Cu	Co
10	10	1:1	46.6	46.830	49.6	49.700	−0.50	−0.20
20	10	2:1	93.3	93.660	49.9	49.700	−0.38	+0.40
10	20	1:2	47.0	46.830	99.71	99.400	+0.36	+0.30

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Received *June 14th*, 1978
Accepted *August 15th*, 1978

Routine Determination of Nitrogen by Kjeldahl Digestion Without Use of Catalyst

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Keywords: Nitrogen determination; non-toxic Kjeldahl digestion; hydrogen peroxide oxidation

The use of a catalyst in Kjeldahl digestion has been widely examined; its presence substantially accelerates oxidation and completes the digestion to allow the subsequent determination of nitrogen. Results from many investigations have been reported by Bradstreet.[1] These results have established mercury as being the most effective catalyst and it is universally used in routine laboratory work. However, it is very toxic and expensive to use, and consequently much research has been undertaken to find an efficient substitute of low toxicity and cost.

Stirrup and Hartley[2] and Williams[3] used copper(II) sulphate in combination with non-toxic titanium dioxide and found this mixture to be a suitable replacement for mercury in the analysis of feedingstuffs and cereal grains. Glowa[4] tested non-toxic zirconium dioxide alone and also combined with copper(II) sulphate as a possible replacement for the mercury catalyst used in the AOAC[5] standard method for the analysis of feedingstuffs and fertilisers. A longer clearing period in the initial stage of digestion was required for zirconium dioxide when used alone and consequently the use of a mixed catalyst was recommended. Tingvall[6] used a copper catalyst with high-temperature digestion at 405 °C; good recoveries of nitrogen from amino acids were obtained. Mercury can be replaced by other catalysts provided that certain parameters of the digestion procedure are modified. These parameters may be the extension of the digestion period, the elevation of boiling-point by appropriate adjustment of salt to acid ratio and the use of temperature-controlled apparatus. However, the use of a pollution-free digest for the determination of nitrogen in the routine laboratory is very desirable. Trials have been undertaken to establish an oxidation scheme yielding such a digest with the use of hydrogen peroxide.

Bradstreet[1] has reported on the use of hydrogen peroxide without a catalyst in the macro-digestion of samples taken from a variety of products. The digestion time is dependent on the type of product and large volumes of hydrogen peroxide solution and sulphuric acid are used. These requirements are not compatible with routine analysis. A recent paper by Tomonari *et al.*[7] has described the use of hydrogen peroxide without a catalyst for the determination of nitrogen in feedingstuffs. The results obtained were in good agreement with those from a standard method, except for fishpaste, and a bad recovery of nitrogen from tryptophan was reported.

In this paper a procedure is described for the routine determination of nitrogen in feed-stuffs and biological products on a semi-micro scale by use of Kjeldahl digestion but without the use of a catalyst. Multiple additions of hydrogen peroxide solution are made in order to accelerate decomposition and clarification. Digestion for a further 30 min gives complete conversion. Nitrogen in the digest is determined by using an AutoAnalyzer and the results are processed by a computer.

Experimental

Apparatus

Digestion tube. These were 150 × 16 mm in size, rimless, Pyrex and calibrated at 25 ml.

Digestor. This was an electrically heated micro-Kjeldahl range, holding 24 tubes (Gallenkamp, London).

AutoAnalyzer. A Technicon Mark I analyser (Technicon, Basingstoke).

Datalogger. A Venture model (Digitronix Ltd., Milton Keynes).

Computer. A PDP 11/10 (Digital Equipment, Galway, Ireland).

Reagents

Anti-bumping granules.

Hydrogen peroxide solution, 30% m/V, analytical-reagent grade.

Sulphuric acid, d = 1.84, *analytical-reagent grade.*

Potassium sulphate tablets, each weighing 1.2 g. These were obtained from Thompson and Capper Ltd., Cheshire.

Mercury catalyst tablets, each containing 0.05 g *of red mercury(II) oxide and* 1.2 g *of potassium sulphate.* Kjeltabs FM (Thompson and Capper Ltd.) were used.

Procedure

Preparation of sample

Feedingstuffs were ground to pass through a 1 mm screen aperture in an 8-in laboratory hammer mill (Christy and Norris Ltd., Chelmsford). Acidified faeces were homogenised and samples for analysis drawn out by use of a syringe fitted with a wide-bore needle.

Digestion

A 0.1-g amount of dried sample was weighed directly into a tube. A 1-g sample was taken in the instance of acid faeces and 1 ml was taken by pipette for other liquid samples. A few anti-bumping granules were added.

Test method

One tablet of potassium sulphate was added to the sample, followed by 3 ml of sulphuric acid. The tube was then placed on the range without any application of heat. The tube was supported at an angle of approximately 45° on this range and was completely visible to the operator. Approximately 1 ml of hydrogen peroxide solution was added by using a Pasteur pipette and the reaction allowed to take place at ambient temperature. When this reaction was completed (2–3 min) the tube was heated and the rate of digestion controlled by selecting appropriate heat settings. Low and high settings are provided for the heating spiral at the bottom of the tube and a high setting only for the spiral at the side-wall of the tube. Hydrogen peroxide solution was then added one drop at a time by means of the Pasteur pipette. These additions were made carefully, allowing the drop to trickle down the inside of the wall of the tube and to flow slowly into the acid mixture, thereby minimising the effervescence. It was important to allow this reaction to be completed for each drop before making a further addition as the reaction can be violent and dangerous when an excessive volume of peroxide is added. Additions were continued until the clearing stage was completed. This object was achieved when the digest became permanently discoloured and white acid fumes appeared. No further use of hydrogen peroxide was made. Digestion was continued for 30 min in order to complete the process.

Standard method

One tablet of mercury catalyst was added to the sample followed by 3 ml of sulphuric acid. The tube was heated on the range with the rate of digestion controlled as in *Test method.* Digestion was continued for 1 h after clearing.

Autoanalysis

The digest was diluted to 25 ml with de-ionised water and an aliquot analysed to determine nitrogen by using the method described on Technicon Method Sheet N-3b. Ammonium sulphate solutions were used for calibration. The presence of mercury increases the formation of indophenol dye and this enhancement of colour intensity has to be compensated for as described by Davidson *et al.*[8] Consequently, standard solutions for calibration (see *Standard method*) contained 2 mg of mercury(II) oxide per millilitre of solution.

Data processing

The signal response for peak height was recorded on a cassette tape by datalogger and this tape fed to the programmed computer. Calibration standards were run both at the

beginning and at the end of the run in order to make a correction for base-line drift, assuming that this drift was constant with time.

Results and Discussion

Preliminary investigations were carried out to determine the digestion time required by the test method using a sample of white fishmeal.[1] Results are given in Table I. Analysis by use of the standard method gave a nitrogen content of fishmeal[1] of 10.10 ± 0.10%. It is evident that a 30-min period following clarification is adequate. This period was adopted for all further digestions by the test method.

TABLE I

DIGESTION TIMES FOR NITROGEN DETERMINATION IN WHITE FISHMEAL[1] BY USE OF THE TEST METHOD

Time after clearing/ min	Nitrogen,* %	Standard deviation, %
30	10.21	0 07
45	10.17	0.07
60	10.09	0.06
75	10.18	0.05

* Mean of four replicates.

Samples taken from a variety of feedingstuffs and biological materials were analysed in triplicate by both test and standard methods. The results are given in Table II. There is good agreement for all of the products.

TABLE II

NITROGEN DETERMINATION IN FEEDINGSTUFFS AND BIOLOGICAL PRODUCTS BY TEST AND STANDARD METHODS

	Test method		Standard method	
Sample	Nitrogen, %	Mean, %	Nitrogen, %	Mean, %
Barley	1.87, 1.86, 1.85	1.86	1.87, 1.84, 1.85	1.85
Hay	1.43, 1.46, 1.45	1.45	1.41, 1.49, 1.39	1.43
Grass meal	1.87, 1.82, 1.80	1.83	1.82, 1.85, 1.84	1.84
Concentrates	2.48, 2.47, 2.45	2.47	2.47, 2.44, 2.48	2.46
Weatings	2.33, 2.33, 2.36	2.34	2.37, 2.37, 2.37	2.37
White fishmeal[2]	10.44, 10.64, 10.64	10.57	10.54, 10.66, 10.62	10.61
Skim-milk powder	5.25, 5.48, 5.49	5.41	5.30, 5.36, 5.42	5.36
Whole milk	0.592, 0.572, 0.588	0.584	0.587, 0.596, 0.606	0.596
Urine (cow)	0.528, 0.528, 0.529	0.528	0.521, 0.528, 0.526	0.525
Acidified faeces (cow)	0.152, 0.151, 0.152	0.152	0.154, 0.149, 0.150	0.151

Digestion by means of the test method was further examined by measuring the recovery of nitrogen from tryptophan, which is considered a good reference standard for assessing the efficiency of digestion. Results for both the test and standard methods are given in Table III. These results compare well for both accuracy and precision.

TABLE III

RECOVERY OF NITROGEN FROM TRYPTOPHAN BY TEST AND STANDARD METHODS

		Nitrogen, %		
	Number of analyses	Mean	Standard deviation	Recovery, %*
Test method	14	13.55	0.29	98.8
Standard method	15	13.59	0.35	99.1

* Theoretical value of 13.72%.

The method described in this paper is most suitable for carrying out routine laboratory work, where one operator can work four digestion units (96 determinations) simultaneously. The stages of digestion are staggered, with each unit employed in a cycle of events as follows: initial cold reaction with hydrogen peroxide solution; preliminary clearing with hydrogen peroxide solution; final clearing with hydrogen peroxide solution; completion of digestion without the use of hydrogen peroxide solution. This cycle permits the operator to handle a maximum of two units at one time for the additions of hydrogen peroxide solution to hot digest.

When using the method frothing was minimised by the continuing reaction of hydrogen peroxide, and any residue on the side-wall of the tube was easily washed down by the small additions of hydrogen peroxide solution. The initial clearing stage was rapid and the total digestion time averaged 60 min for the products tested. The cost of reagent chemicals required for a single digestion by test and standard methods was 2.1 and 0.9 p, respectively. The extra cost of digestion by use of the test method can be offset by the cost of labour and equipment required for the recovery of mercury from digest solutions obtained when using the standard method in order to prevent environmental pollution. Calculation of the results by computer was fast, reliable and accurate.

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Received *August 14th*, 1978
Accepted *October 26th*, 1978

Book Reviews

HIGH PERFORMANCE MASS SPECTROMETRY: CHEMICAL APPLICATIONS. By MICHAEL L. GROSS. *A symposium co-sponsored by the University of Nebraska, Lincoln, the National Science Foundation, AEI Scientific, and INCOS Corp., Lincoln, November* 3–5, 1976. *ACS Symposium Series No.* 70. Pp. x + 358. Washington, D.C.: American Chemical Society. 1978. Price $28.

The objective of the book is to survey current research in high-performance mass spectrometry. It is divided into two sections, the first representing about a quarter of the book. This describes methods for acquiring metastable ion data which relate to stable and low energy decomposing ions and the application of such methods to determine ion structures, properties and potential energy surfaces. Methods for investigating very rapid ion reactions using field ionisation kinetics are also covered.

The second and much longer part of the book considers the use of high-performance mass spectrometry for tackling analytical problems. It covers both qualitative and quantitative mass spectrometry, using a variety of ionisation techniques, including simultaneous positive and negative ion chemical ionisation. Direct inlet and GC - MS are covered. For those who regard GC - MS as the only method for the analysis of organic mixtures, the chapter on trace analysis by evaporation from the direct inlet probe and detection by single ion monitoring is a useful reminder that this approach can be a valuable additional method. As a vehicle for demonstrating this, the author describes the analysis of a number of commodities for trace amounts of aflatoxins.

GC - MS is, of course, vital for many applications and the subsequent chapter gives an introduction to the technique. The particular considerations for GC with high-resolution mass spectrometry are noted, and the relevance of selecting appropriate GC conditions is considered.

In one of the chapters on chemical ionisation mass spectrometry, a method for the generation of exact mass data using a quadrupole instrument is given.

The use of field desorption with chemical ionisation, and in a later chapter with electron impact, is described.

The use of various forms of mass spectrometry in biomedical applications is well presented, with some examples of selected ion monitoring for the detection of drug metabolites in urine extracts. A well balanced chapter on field desorption helps to distinguish myth from fact and is backed by examples of biomedical applications.

Pyrolytic fragmentation is an effect which spectrocopists often try to avoid. There are occasions when deliberate pyrolysis coupled with electron-impact fragmentation can be put to advantage, and one paper describes such a procedure for identifying some nucleosides in DNA.

The value of ultra-high-resolution measurements in the analysis of petroleum products is demonstrated, and emphasises the need for such a procedure combined with high-performance liquid chromatography. There is only one paper on inorganic mass spectrometry, and the use of isotope dilution for trace analysis.

The last two chapters of the book emphasise the need for a computer system in order to make full use of the vast amount of data that is generated per unit time by a mass spectrometer.

The book represents a useful collection of good quality papers and is certainly recommended reading to those concerned with the analytical aspects of mass spectrometry. Presumably in some attempt to imply rapid publication, all of the contributions are dated December 30th, 1977. In fact, the contributions were presented at a symposium in November, 1976, and the manuscripts have been up-dated. Would it not have been more useful to have published the original papers much sooner?

T. A. GOUGH

TRACE ELEMENTS IN HUMAN HAIR. By VLADO VALKOVIC. Pp. x + 194. New York and London: Garland STPM Press. 1977. Price $19.

Hair is a complex biological material. Amongst its interesting properties is the ability along with other keratinous materials to concentrate trace elements in its structure. Hence the potential exists for studying the recent past of a person in terms of the distribution of these elements along the shaft of a growing hair. Any book giving information on this topic is therefore to be welcomed, but in fact reading "Trace Elements in Human Hair" provokes very mixed feelings. The positive contributions are offset by some serious deficiencies.

There are chapters on hair growth; structure of hair; trace elements in hair; the role of trace elements; applications of trace-element measurements; and methods for measurement of trace-element levels in hair. There is also a comprehensive set of references, but unfortunately this only covers the period up to 1975, with few references from 1976 and later. Because of this, most of the work described in the book on the quantitative analysis of hair is concerned with "bulk" samples, which can give only an average result for a particular person. More recent studies, *e.g.*, that by Maes and Pate, *J. Forens. Sci.*, 1976, **21**, 127, have shown that frequently there is as much variation in composition between hairs taken from different parts of the head of one person as there is between different people. Hence a more critical examination by the author is needed, particularly in assessing the significance of the results which he reports from the many research workers in this field.

The chapter on methods of measurement deals exclusively with the techniques available a year or so ago, *viz.*, atomic-absorption spectroscopy, neutron-activation analysis, X-ray fluorescence emission induced by proton beams or radioactive sources, and spark-source mass spectrometry. It would have been worthwhile enlarging this section to include firstly electron probe work, particularly with plasma-ashed samples and using wavelength-dispersive analysers to increase the signal to noise ratio, and secondly fine-beam X-ray fluorescence methods.

The book contains a number of factual errors. For example, the statement "hair not dependent on *steroid hormones* . . . but sometimes inhibited by *androgenic hormone*" immediately suggests the riddle, when is a steroid not a steroid?

Typographical errors abound. A cursory check revealed over 40 misspellings; the absence of adequate proof reading, even though the book appears to have been produced from typescript, is quite inexcusable.

R. L. WILLIAMS

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISK OF CHEMICALS TO HUMANS. Volume 17. SOME *N*-NITROSO COMPOUNDS. Pp. 365. Lyon: International Agency for Research on Cancer. Distributed by the World Health Organization. Available in the UK through HM Stationery Office. 1978. Price SwFr50; $25.

The Monographs were begun in 1972 in order to collect all available relevant experimental and epidemiological data on chemicals to which man is exposed and to assess the data from the point of view of risk. The working group consists of experts in the various appropriate fields, notably pathology, microbiology, toxicology, physiology, chemistry and epidemiology. A new group, frequently with different contributors, is convened for each monograph.

The present collection of monographs is devoted exclusively to *N*-nitroso compounds, some of which have been discussed in earlier publications in this series (Volumes 1 and 4). A vast amount of work on these compounds has been undertaken in the last few years and the appearance of this book, updating and expanding the earlier volumes, is most welcome.

The first part of the volume describes the background to the series, the objectives and scope and some comments on the selection of chemicals. The general principles for evaluating the carcinogenic risk of the selected chemicals is described. This includes an assessment of the various aspects of the published data based on animal experiments, any evidence of carcinogenicity in humans and epidemiological data. The second part of the book consists of a monograph on each of 17 *N*-nitroso compounds. This includes the simple aliphatic nitrosamines, and some heterocyclic nitrosamines which have been the subject of many studies on their occurrence in foodstuffs. *N*-Nitrosodiethanolamine and *N*-nitrosomorpholine, which have been found in industrial chemicals and formulations, are included, as is *N*-nitrosonornicotine, which appears to be unique to tobacco products. There are also several nitrosoamino acids and nitrosofolic acid, the latter apparently prepared only as a laboratory experimental chemical. Two nitrosoalkylureas, which have been used for many years for the laboratory preparation of the corresponding diazo compounds, are listed. Their use for this purpose is no longer encouraged, and one has been studied for use as a cancer chemotherapeutic agent. One nitroso compound (steptozotocin) occurs naturally and is formed microbiologically, and has application in the study of diabetes.

For each of the above compounds the following information is given, where available:

1. synonyms, trade-names, molecular formula and basic physical properties;
2. production, both in the laboratory and commercially, and uses, which for this group of compounds are very restricted;

3. occurrence, which is a particularly interesting section with respect to the nitrosodialkylamines as they have been found in food, water, air, industrial chemical formulations, tobacco smoke and drugs; *N*-nitrosodimethylamine is by far the most common and a detailed list of levels of occurrence in foods is tabulated; this nitrosamine is also formed *in vivo* and there is some evidence for its occurrence in human biological fluids;
4. analysis, which is very brief as the IARC has recently published a separate monograph covering the volatile nitrosamines,[1] and there are few methods for the remaining compounds;
5. biological data, only included if they are relevant to the assessment of risk, and normally covering carcinogenicity and related studies in animals; observations on humans are included in this section although in most instances no data are available;
6. the final section of each monograph summarises the data and attempts to make an evaluation, clearly a most difficult task.

Each monograph has a set of references from which the source material was obtained. The book, although a paperback, is well presented and no errors were detected. It is commendably up to date and was published in May, 1978, only 7 months after the Working Group had met.

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T. A. GOUGH

ION-SELECTIVE ELECTRODES. CONFERENCE HELD AT BUDAPEST, HUNGARY, 5–9 SEPTEMBER 1977. Edited by E. PUNGOR and I. BUZÁS. Pp. x + 613. Amsterdam, Oxford and New York: Elsevier. Budapest: Akadémiai Kiadó. 1978. Price $75; £45.45; Dfl180.

This volume arrived for review hard on the heels of that from the conference held a year earlier in Budapest (see *Analyst*, 1978, **103**, 1007). Again we have camera-ready copy of variable quality but a much fatter volume with a more than commensurate increase in price, which one suspects is not unrelated to the distribution in the West having been taken over by an international house.

There are the texts of seven Plenary Lectures, occupying about one third of the book. Like the so-called discussion lectures (there is no discussion included), these are arranged in the book in alphabetical order of the first named contributor. Some gathering together under topic headings would have been helpful to the reader. Some of the Plenary Lectures (Bates, Dolidze) present new work, others review their own or others'. Pungor's lecture, placed sixth, is more in the nature of an introduction to current problems.

The discussion lectures fall into the categories of mechanistic studies (Baucke, Koryta, Boksay, Belijustin, Nagy), analytical usage (the majority) and clinical (Durst, Fuchs, Havas, Mascini). It may be noted that six of the papers are on pH glass electrodes, of which the most notable is that of Baucke, who reviews his work on the ion-sputtering technique of studying concentration profiles in surface layers and presents a new mechanism based on silanol group dissociation. Other papers presenting significant advances are those of Nagy, Fjeldly and Johannessen, who show that there is a fluoride depleted layer on the surface of the lanthanum fluoride electrode from Auger spectroscopy, Tacussel and Fombon, who discuss new phosphate and chloride sensors, and Neshkova and Sheytanov, who describe plated chalcogenide electrodes.

In short, a mixed bag of a more international flavour than at the previous meeting, which all interested in ISE's will need to borrow from their institution's library; it could only be recommended for private bookshelves at half the price.

A. K. COVINGTON

Analysis 79

AUTOMATION IN INDUSTRIAL AND CLINICAL CHEMISTRY

16 – 18 July, 1979

The City University, Northampton Square, London, EC1

Workers in clinical industrial and academic environments will find this conference of particular value as it has been designed to provide a cross-fertilization of the ideas and concepts of automation between the three sectors. Not only scientific and technical aspects will be considered, but also managerial, organisational and economic considerations which are an important part of the application of automation. The broad areas of the conference will be: **education, new instrumentation, costing and management, new applications, evaluation and standardization**

KEYNOTE SPEAKERS

Professor M Bonner Denton, The University of Arizona, USA
Dr F L Mitchell, Clinical Research Centre, Harrow, Middlesex, UK.
Dr R W Arndt, Mettler Instruments AG, Griefensee, Switzerland.

Some of the papers included in the programme

Training of clinical laboratory personnel in the use and maintenance of automatic systems, **Dr L B Roberts,** Gartnavel General Hospital, UK

An experiment in education for automatic analysis: the 1979 Chemical Society Summer School, **Dr D Betteridge,** University College of Swansea, UK.

Case studies in laboratory automation, **Professor M Bonner Denton,** The University of Arizona, USA.

Recent developments in flow injection analysis, **Dr J Ruzicka,** the Technical University of Denmark, Lyngby, Denmark.

DACOS – a new approach to kinetic analysis, **M Snook,** Clinical Research Centre, Harrow, Middlesex, UK.

Recent developments in automatic chromatography, **Dr P B Stockwell,** The Laboratory of the Government Chemist, London, UK.

Automated method for determination of sulphate in water, **M Stockley,** Yorkshire Water Authority, and **R J Vincent,** Thames Water, UK.

The cost benefits of automated analytical systems, **J G Jones,** Wessex Water Authority, Bath, UK.

Economic techniques for evaluating automation alternatives, **T M Craig,** E I Du Pont de Nemours & Co, Wilmington, Delaware, USA.

Evaluation of clinical laboratory equipment, **Dr L B Roberts,** Gartnavel General Hospital, Glasgow, UK.

Automation of radioimmunoassay and related analytical techniques, **Professor J Landon,** St Bartholomew's Hospital, London, UK.

Industrial applications of automation with particular reference to the InfraAlyzer, **Dr H Swann,** University of Nottingham, School of Agriculture, Sutton Bonnington, Loughborough, UK.

Extraction in continuous flow systems with examples from pharmaceutical analysis, **Dr B Karlberg,** Astra Pharmaceuticals AB, Sodertalje, Sweden.

Improved accuracy in automated chemistry through the use of reference materials, **Dr R F Coleman,** National Physical Laboratory, Teddington, UK.

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Reproducibility of Pyrolysis - Mass Spectrometry Using Three Different Pyrolysis Systems

A study has been made into some of the factors that affect the reproducibility of pyrolysis - mass spectrometry. Three separate pyrolysis systems were examined and three sample types, a simple system of easily pyrolysable polymers, an acrylic paint, and an alkyd paint, were employed in order to cover a range of ease of sample pyrolysis. These samples were also examined by pyrolysis - gas chromatography.

The reproducibility of the pyrolysis - mass spectrometry system was found to vary according to sample type. The source of irreproducibility was identified as the pyrolysis process and not the mass spectrometric detection.

Keywords: *Reproducibility*; *pyrolysis - mass spectrometry*; *pyrolysis - gas chromatography*; *paint analysis*

D. A. HICKMAN and I. JANE

Metropolitan Police Forensic Science Laboratory, 109 Lambeth Road, London, SE1 7LP.

Analyst, 1979, **104**, 334–347.

Interference Films on the Sensor Membranes of Solid-state Copper(II) Ion-selective Electrodes

Copper(II) ion-selective electrodes based on copper(II) sulphide - silver sulphide sensor membranes, which showed anomalous responses with copper(II) nitrate in the presence of chloride, have been examined by Auger spectrometry. In some electrodes exposed to solutions of potassium chloride the chloride is found to have penetrated the bulk of the membrane matrix, whilst in others only a surface contamination is observed. The anomalous electrode response is exhibited when exposure to chloride is in the presence of copper nitrate. The Auger signal alters during the duration of the spectrum as a consequence of electron bombardment. The effects of argon-ion and electron bombardment are compared.

Keywords: *Ion-selective electrodes for copper(II)*; *interference films on ion-selective electrodes*; *Auger spectrometry*

G. J. MOODY, N. S. NASSORY and J. D. R. THOMAS

Chemistry Department, University of Wales Institute of Science and Technology, Cardiff, CF1 3NU.

D. BETTERIDGE, P. SZEPESVARY and B. J. WRIGHT

Chemistry Department, University College of Swansea, Singleton Park, Swansea, SA2 8PP.

Analyst, 1979, **104**, 348–357.

Determination of Nitrite Ion in Unused Cutting Fluids and Cutting Oils Using a Gas-sensing Electrode Method

Modifications of the Orion NO_x gas-sensing electrode method that were made to determine nitrite ion in unused cutting fluids and cutting oils are described. Detection limits for both types of lubricants of the order of 15 $\mu g\,g^{-1}$ of NO_2^- ion were obtained. Previous analysis of six cutting fluids, collected in the Ottawa region and analysed by spectrophotometry, confirmed the presence of high levels of nitrite ion and showed fair agreement between results. Analysis of six other cutting fluids and 20 cutting oils collected in the same region showed the presence of nitrite ion in only three instances. The operation of the electrode, interferences, the use of standard-addition and -subtraction methods and the possibility of applying this method to used cutting lubricants are discussed.

Keywords: *Nitrite-ion determination*; *gas-sensing electrode*; *nitrogen oxide electrode*; *cutting fluids*; *cutting oils*

FERRERS R. S. CLARK and HART B. MACPHERSON

Product Safety Laboratory, Department of Consumer and Corporate Affairs, Tunney's Pasture, Ottawa, Ontario, Canada, K1A 0C9.

Analyst, 1979, **104**, 358–366.

Dicarboxidine [γ,γ'-(4,4'-Diamino-3,3'-biphenylenedioxy)dibutyric Acid] as a Reagent for the Spectrophotometric Determination of Cyanide and Chlorine

Short Paper

Keywords: Dicarboxidine [γ,γ'-(4,4'-diamino-3,3'-biphenylylenedioxy)dibutyric acid] chromogen; cyanide determination; chlorine determination; spectrophotometry

KERSTIN GRÖNINGSSON

Research Department, Analytical Chemistry, AB KABI, S-112 87 Stockholm, Sweden.

Analyst, **1979**, **104**, 367–370.

Semi-automatic Determination of Manganese in Natural Waters and Plant Digests by Flow Injection Analysis

Short Paper

Keywords: Manganese determination; water analysis; plant material analysis; flow injection analysis; spectrophotometry

M. F. GINÉ, E. A. G. ZAGATTO and H. BERGAMIN FILHO

Centro de Energia Nuclear na Agricultura, CEP 13400 Piracicaba, São Paulo, Brazil.

Analyst, **1979**, **104**, 371–375.

Gravimetric Determination of Copper(II) and Cobalt(II) by Selective Precipitation with Benzimidazole

Short Paper

Keywords: Copper determination; cobalt determination; benzimidazole; gravimetry

K. N. UPADHYAYA

Chemistry Department, University of Dar es Salaam, P.O. Box 35061, Dar es Salaam, Tanzania.

Analyst, **1979**, **104**, 375–377.

Routine Determination of Nitrogen by Kjeldahl Digestion Without Use of Catalyst

Short Paper

Keywords: Nitrogen determination; non-toxic Kjeldahl digestion; hydrogen peroxide oxidation

ERIC FLORENCE and DOUGLAS FRANK MILNER

National Institute for Research in Dairying, Shinfield, Reading, RG2 9AT.

Analyst, **1979**, **104**, 378–381.

ANALYTICAL SCIENCES MONOGRAPHS No. 3

Pyrolysis–Gas Chromatography

by R. W. May, E. F. Pearson and D. Scothern

This monograph attempts to present the available knowledge in a form useful to the practising analyst, helping in the choice of an appropriate method and in the avoidance of the more common pitfalls in this, perhaps deceptively, simple technique.

Chapter 1 serves as an introduction to gas chromatography and will be of interest to those unfamiliar with the technique. The several methods of pyrolysis used in pyrolysis–gas chromatography are described in Chapter 2; their merits and demerits in particular applications are discussed. The major analytical uses of the technique are presented in Chapter 3; the general analytical 'fingerprinting' aspects described separately from the method as applied to specific sample types. Chapter 4 deals with the identification of the pyrolysis products which are eluted from the chromatography column, useful extra information allowing the possibility of naming a pyrolysed sample without recourse to a known identical sample. The necessity for increased standardization of the technique of pyrolysis–gas chromatography is discussed in Chapter 5.

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by C. W. Fuller

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Printed by Heffers Printers Ltd Cambridge England
Entered as Second Class at New York, USA, Post Office

25.WR.2522