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A Review

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ERIC REID

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Analyst, 1976, **101**, 1–18.

The Automatic Determination of Silicate Dissolved in Natural Fresh Water by Means of Procedures Involving the Use of Either α- or β-Molybdosilicic Acid

This paper describes the practical application of Truesdale and Smith's (1975) fundamental re-appraisal of the conditions that lead to the formation of α- and β-molybdosilicic acid in aqueous solutions. Two procedures, one using the α-acid and the other the β-acid, are proposed. Both procedures are designed so as to enable silicate-silicon concentrations of between 0 and 1·0 mg l^{-1} to be determined, although slight adjustments to the manifold of the Technicon AutoAnalyzer II system would make both suitable for other concentration ranges. The appropriate "molybdenum blues" derived from the yellow acids are used.

Tests of the precision of both procedures were made at five concentration levels between 0 and 1·0 mg l^{-1} of silicate-silicon in distilled water. The maximum value of the coefficient of variation was 1·44 per cent. In other tests 27 samples of natural water from the upland area surrounding the sources of the rivers Wye and Severn were analysed by both methods. The results suggest that for these types of water the methods yield the same value for silicate concentration.

VICTOR W. TRUESDALE and CHRISTOPHER J. SMITH

Institute of Hydrology, Maclean Building, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB.

Analyst, 1976, **101**, 19–31.

The Determination of Strontium-90 and Strontium-89 in Water without Separation of Strontium from Calcium

A method has been developed for measuring strontium-90 and strontium-89 activities in water without separating the strontium from calcium. After evaporation of the sample to a convenient volume, the strontium plus calcium is purified radiochemically and an aqueous solution of their chlorides counted in a liquid scintillation spectrometer, which records the Čerenkov radiation produced. After a suitable period so as to allow the ingrowth of yttrium-90, the source is re-counted. From the two counts the activities of both strontium-90 and strontium-89 are calculated.

Activities as low as 0·1 pCi l^{-1} in natural waters as well as the higher levels in effluents from nuclear installations can be measured by this method.

J. G. T. REGAN and J. F. C. TYLER

Department of Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

Analyst, 1976, **101**, 32–38.

The Determination of Tellurium in Leaded Free-cutting Steels by Atomic-absorption Spectrometry

A procedure is presented for the determination of tellurium within the range 0·005–0·08 per cent., with solvent conditions that permit its incorporation in a composite scheme that includes lead. In the preferred fuel-rich flame the background absorbance of iron and inter-element interferences are at a minimum.

Good recoveries of tellurium when added to solutions of leaded and stainless steels, together with satisfactory reproducibilities for samples and good agreements with results by the slower turbidimetric procedure, are shown.

W. D. COBB, W. W. FOSTER and T. S. HARRISON

British Steel Corporation, Scunthorpe and Lancashire Group, P.O. Box No. 1, Scunthorpe, South Humberside, DN16 1BP.

Analyst, 1976, **101**, 39–43.

Colorimetric Determination of Phosphorus in Silicates Following Fusion with Lithium Metaborate

The determination of phosphorus in silicate materials by use of a colorimetric technique following fusion with lithium metaborate is described. The method is rapid and the results obtained compare favourably with the accepted values for standard rock and mineral samples and also with gravimetric results. Reliable values of up to 1·75 per cent. for the phosphorus(V) oxide content have been obtained by this method.

J. B. BODKIN

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Analyst, 1976, **101**, 44–48.

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Sample Preparation in the Micro-determination of Organic Compounds in Plasma or Urine

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Introduction

When trace amounts of therapeutic substances or of endogenous compounds of comparatively low relative molecular mass have to be determined, possibly as metabolites, an isolative procedure of variable complexity usually has to be applied to the sample initially, whether it is of plasma or urine. As listed for drugs[1] the concentrations of the organic substances may vary from 10 to less than 10^{-1} mg l^{-1}. The portion of sample taken is typically 1 ml for plasma samples and 10 ml for urine, although the latter may be richer in the substance(s) to be assayed. Removal of adventitious fluorogens may be necessary with plasma (*e.g.*, reference 2) and especially with urine (*e.g.*, reference 3). In general, the sample has to be partially purified.

These generalisations do not apply to radioimmunoassay, which is not to be considered here, although it is notable for its high sensitivity, if not assured specificity,[4] and is of growing importance in drug assay.[4,5] In fact, a sample preparation step may be desirable even for radioimmunoassay (*e.g.*, reference 6) and other affinity (saturation analysis) methods (*e.g.*, references 7 and 8).

General guidance on ways of preparing samples for assay is scarce in the literature and the *rationale* for particular methods is often unstated. A classical paper by Brodie *et al.*[9] on the assay of basic substances repays study, as do articles by Titus[10] and Trevor *et al.*[11] and a book on separation science.[12] The present survey of approaches is prompted by our analytical involvement in bioavailability trials and in method-development studies on metadrenalines (metanephrines), *i.e.*, 3-*O*-methyl derivatives of catecholamines. Some examples that illustrate principles have been taken from the latter field as well as the literature on drug assay.

* Reprints of this paper will be available shortly. For details see summaries in advertisement pages.

Toxicological literature, concerned with the screening of urine or blood samples for drugs of abuse, is largely unhelpful, partly because the drug concentrations are often high.[1] The emphasis has often been more on rapid detection than on exact determination, and indeed many papers lack results on percentage recovery. Moreover, a multi-purpose preparation procedure [*e.g.*, repeated extraction with a mixture of acetone and diethyl ether (1 + 1)[13]] has commonly been favoured, although with awareness of its possible inefficiency for some of the drugs that might be present.

Ionisability and Other Chemical Features

Drugs and other organic compounds that are determined in body fluids may be non-ionisable (*e.g.*, glutethimide and sulphonylureas), anionic except below a certain pH value (*e.g.*, barbiturates and aspirin) and cationic except when dissociated at alkaline pH (*e.g.*, morphine and catecholamines). Alternatively, the classes can be termed acidic, basic and inherently neutral. The term acidic usually connotes acidity greater than that of phenolic hydroxyl groups. Amphoteric compounds comprise a special class, having a charged group virtually irrespective of the pH.

In contrast with ion-exchange chromatography, solvent extraction is best performed with compounds in un-ionised form, taking account of differences in polarity that govern the choice of solvent (see Development of Solvent Methods, below). Whatever the approach, the object of the analytical exercise will be frustrated if interfering compounds are carried through to the final step, or if autoxidation is allowed to occur. For phenolic compounds, which in alkaline solution are especially prone to autoxidation, 2-mercaptoethanol is a favoured antioxidant,[14,15] although it can jeopardise the use of fluorimetry.

Risk of Adsorptive Losses

The following remarks are concerned not with possible chromatographic losses due to incomplete elution, as referred to below (in the section on Separation with a Solid Additive or Column Material), but with possible adsorption of the sample on to cuvettes or other vessels in micro-determinations. This phenomenon, perhaps better known in the context of very dilute solutions of purified proteins, can lead to erratically low assay values (*e.g.*, reference 16). While the presence of a detergent helped to obviate this trouble in the instance of tetrahydrocannabinol,[6] a more common preventive measure is to silanise the glassware or to apply a very thin coat of PTFE.[17] Such adsorption, as recognised by Brodie *et al.*,[9] can occur both with aqueous media, particularly at neutral or alkaline pH values for organic bases, and with organic solvents, especially if of weak polarity. The adsorptive loss they observed with chloroquine at a concentration of 70 μg l^{-1} in heptane had risen to 25 per cent. after 30 min, but was nil if ethanol had been added (to 1·3 per cent. *V/V*). In extracting quinacrine with light petroleum,[18] they used isoamyl alcohol (mainly, if not entirely, 3-methylbutan-1-ol*) to minimise adsorption. In sampling from heptane extracts, Dill *et al.*[19] used pre-wetted pipettes. Material lost on to extraction tubes may re-appear in subsequent assays if the tubes are re-used, as was observed by Spirtes[20] with chlorpromazine; silanisation did not prevent this problem, but treatment with acidic dichromate and ammonia solution did do so. Use of a scavenger may also help.[17]

Inadvertent adsorption is not a universal occurrence. In scintillation counting studies the adsorption of benzoic acid was found to be small, at least with present-day vials, compared with that of dicarboxylic acids.[21] With oestrogens in aqueous buffers there were losses due not to adsorption but to creepage; these could be minimised by silanisation.[22]

Initial Treatment of Plasma Samples

When blood rather than urine is the starting material there are special problems, which will only be touched on here. With serum, U.S.-manufactured devices to aid separation from the clot may give rise to drug-like analytical artifacts.[23,24] Compounds that are present in samples of plasma (or serum, which is virtually synonymous in the present context) normally have to be obtained free from protein, yet without loss of any protein-bound moiety. Dis-

* In addition to this isomer, the "isoamyl alcohol" used by many investigators may contain 15–30 per cent. *V/V* of 2-methylbutan-1-ol as stated in some catalogues; however, some suppliers claim high purity.

sociation of the latter may be favoured by diluting the plasma. For this and other reasons, five-fold dilution of the plasma at the outset is advantageous.[11]

Overnight ultrafiltration, as in an assay method for acetaminophen (paracetamol),[25] is a possible way of deproteinising the plasma, although it would not itself encourage dissociation of a bound drug. A more common way is to use an acidic protein precipitant, *e.g.*, tungstate followed by sulphuric acid.[3] Some workers[14] use perchloric acid, added to give a concentration of 5 per cent. m/V; this acid has the advantage that the perchlorate anion can, if desired, be removed subsequently as its insoluble potassium salt. Trichloroacetic acid is sometimes used (*e.g.*, reference 9), but it may contain impurities that can give a high assay blank,[14] and it may itself be carried through the assay if the next step is extraction with a solvent such as diethyl ether, in which it is soluble. Nevertheless, it was an effective first step in a gas - liquid chromatographic assay[26] (with an electron-capture detector) for biguanides in plasma at levels as low as 1 μg l^{-1}.

Alternatively, the compound can be extracted from the plasma with an organic solvent, after acidification if it is basic and strongly protein-bound.[9] Indeed, classical protein precipitation procedures are seldom obligatory. They may even be undesirable if the compound is relatively soluble in water (a possible difficulty with heavy-metal reagents being co-precipitation of the test compound with the protein); with use of a suitable pH and, if necessary, with salt addition to the aqueous phase, an effective water-immiscible organic solvent can usually be found, even for coumarin anticoagulants or other drugs that are extensively protein-bound in plasma (A. Bye and R. H. Nimmo-Smith, personal communications). The extraction of serum, half-saturated with sodium dihydrogen orthophosphate, with hexane worked well for methaqualone.[27]

The choice of a water-immiscible solvent is considered later. If 1,2-dichloroethane is used, notwithstanding the risk of a high assay blank,[11] it may be advantageous carefully to layer the plasma on to the solvent, so as to obviate gel formation after mixing.[9] In choosing a suitable pH for the particular compound that is the subject of the assay, it should be remembered that an acidic pH will encourage precipitation of protein. This is particularly desirable when a direct assay is to be carried out on the organic phase, *e.g.*, an isoamyl acetate extract in the colorimetric determination of sulphonylureas[28] and a chloroform extract in the gas - liquid chromatographic determination (after evaporating down the extract) of anti-epileptic drugs.[29] Chloroform extraction at an acidic pH value is effective even for phenytoin, which is strongly protein-bound.[30]

Two examples of water-miscible solvents, which, when present in excess, precipitate the proteins and hopefully liberate the bound compound, are acetone and ethanol.[7,11] These solvents have been used in admixture.[31]

Hydrolysis of Conjugates

With urine, and even with plasma (*e.g.*, reference 25), there can arise the difficulty of the presence of conjugates, particularly glucuronides and sulphates, that should not be neglected in the assay. Conjugates are not amenable to solvent extraction. Approaches that could obviate hydrolysis are considered later (under Methods of Handling Conjugates). Commonly, however, conjugates are first hydrolysed by enzymic means or with hot acid (say 0·1 M hydrochloric acid for 30 min at 100 °C). Acid hydrolysis may give rise to high blanks[14] and poor thin-layer chromatographic patterns,[32] and has to be rigorous for some sulphates and even for some glucuronides[30] (again see Methods of Handling Conjugates). The enzymic approach, possibly with a glucuronidase - sulphatase preparation such as glusulase, is less likely to cause degradation of the compound to be determined or of concomitants, although enzymic treatment can have disadvantages in addition to expense and slowness. Thus, catecholamine assays[15] may be prejudiced by the presence of dopa and dopamine as contaminants in glusulase preparations.[33] Moreover, mucoprotein carried through from the enzyme preparation may cause emulsions or column clogging unless it is removed by an agent such as tungstic acid, and the urine may have to be freed initially from enzyme-inhibiting anions.[14,34]

General Considerations

The processing steps subsequent to any initial splitting-off of bound or conjugated sample compound are the main theme of this review. The detailed processing is governed partly

by the nature of the final analytical step, which is chosen from a range that has been well surveyed by Clifford and Smyth[35] in the context of benzodiazepines. When the final step is not a discriminative analysis, such as differential spectrophotometry, or a separatory approach, such as gas - liquid chromatography,[36] the processing may have to be complex and selective. If, however, a drug to be administered *in vivo* is isotopically labelled, and it is by radioactivity that body-fluid levels of the drug and/or of known metabolites are to be measured, the processing or the final analysis can be less rigorous.

Radioisotopically labelled compounds used *in vitro* rather than *in vivo* are valuable in developing and applying assay methods. When they are added initially in trace amounts to plasma or urine samples, they allow the over-all recovery of the endogenous material in the assay to be established merely by liquid scintillation counting (*e.g.*, reference 32). However, their use can have the disadvantage of necessitating a costly synthesis if they are not available off the shelf. Moreover, if a sample preparation procedure has been devised through the use of radioactivity measurements, and then "real" test specimens (unlabelled) are put through the procedure, the processed sample may prove to be too impure to undergo the classical assay method for determining the endogenous compound.

Traditionally, extra test samples spiked with unlabelled authentic material are put through the sample preparation steps for reference purposes. Separate standards not subjected to the full processing procedure then serve merely to check that parts of the method, for example a final fluorimetric measurement, are working satisfactorily. Processed aqueous standards, which are not actually added to the plasma or urine samples, are of limited value except for pilot work. They may even show a different response to concentration relationship, as observed in a fluorimetric assay for furosemide.[16]

Whatever the calibration procedure, losses as high as 50 per cent. can be tolerated if reproducibility is good. When the final step is chromatographic, the labour of running spiked samples alongside test samples can be obviated by adding to the actual test samples, at the outset, a reference compound that travels in a different position. For example, chloroform has been used for this purpose in the determination of plasma halothane by gas - liquid chromatography.[37] Such a short-cut is invaluable if used warily. Some authors (*e.g.*, reference 26) have taken care to verify the assumed similarity and consistency in respect of processing losses. If the reference compound is merely added just before the chromatographic step its value lies mainly in checking the volume that was chromatographed.

Solvent Extraction

Even in the above assay procedure for halothane, in which plasma can be analysed directly, it is advantageous to perform a traditional solvent extraction with heptane or carbon tetrachloride initially, as by this means the gas - liquid chromatographic column lasts longer and the injection port need not be cleaned so often.[37] However, in most assays a sample preparation procedure is essential rather than optional and solvent extraction is the traditional approach to preparation.[38]

The optimisation of conditions for particular assays is discussed in the section on Development of Solvent Methods. General points to be taken into consideration include solvent purity, as found when using ethyl acetate[39]; inadvertent exposure to air and light rendered the solvent detrimental to the spectrophotometric determination of a catecholamine metabolite (VMA). Chlorinated solvents should be pre-washed with water if phosgene is detrimental to the compound. It is well known that impure solvents may give rise to high blanks.[11,14] Adsorbents such as certain aluminas can constitute an effective means of purifying solvents.[40,41] When specifying isoamyl alcohol, authors should state its composition.

In choosing a solvent, cost is a marginal consideration. For plasma cortisol, chloroform is as satisfactory as dichloromethane and is cheaper.[42] A more important factor is toxicity, which in the case of benzene is so high as to warrant its substitution with toluene. From the viewpoint of laboratory safety, diethyl ether freed from peroxides can be regarded as an acceptable risk. A possible alternative is diisopropyl ether, pre-washed successively with alkali (in order to remove any antioxidant), acid and water; any evaporation of extracts should be carried out in a stream of nitrogen near to room temperature (R. H. Nimmo-Smith, personal communication). Even so, the peroxide hazard is a serious one.

The following remarks mainly concern the equipment needed for the task, especially for tedious multi-step extractions, and practical suggestions.

Extraction Apparatus and Ease of Phase Separation

Traditional separating funnels can be a handicap rather than an asset, particularly when a large number of samples have to be put through successive extractions, each with a centrifugation step. It suffices to extract the samples in stoppered tubes that will withstand centrifugation. If not completely full, they allow efficient extraction when clamped near to the horizontal position and shaken longitudinally (*e.g.*, reference 43) at, say, 120 oscillations per minute for 15 min. It should not be taken on trust that phase mixing (*e.g.*, in an alkane - water system) will be as efficient with a tumbling or rolling device as with vigorous oscillation or even conscientious shaking by hand. Additionally, the possible influence of shaking time, as shown in the extraction of *N*-hydroxyphentermine (for gas - liquid chromatographic assay[44]) and, in our own laboratory, of indomethacin should not be disregarded. Nevertheless Trevor *et al.*[11] give a warning "concerning the time and vigor of extractions which in the reported literature is often far longer than necessary and appears to coincide strongly with the gustatorial habits of a particular laboratory." With plasma, there are instances of the best yield being obtained with less than 1 min of shaking.[38] A roller or tilting device will work well for some solvents and entails a minimum of risk of persisting emulsification.[11,45] Stirring rather than shaking is stipulated if blood is to be extracted with acetone - ether.[13]

Scattered information exists (*e.g.*, reference 11) on the avoidance of emulsions that are intractable to centrifugation. Diethyl ether is less emulsion-prone than chloroform. A recommendation[45] that the organic solvent to aqueous solvent phase ratio be 10:1 cannot sensibly be applied in assay work. A ratio as low as 1:40 gave good extraction of drugs such as amphetamine from urine at an alkaline pH with a dense solvent such as chloroform, obviating the usual need to use a purified solvent and to concentrate the extract.[46] The ratio was only 1:80 in drug-screening studies[47] with a chloroform - propan-2-ol mixture (4 + 1) at an alkaline pH, the urine sample first being saturated with a carbonate - hydrogen carbonate mixture added as a solid; centrifuge tubes with a sharply tapered tip facilitated removal of the heavy organic phase. The authors believed that the addition of salts helped to avoid the formation of emulsions, besides facilitating extraction into a volume of solvent so small that subsequent concentration (for gas - liquid chromatography) was unnecessary. Avoidance of emulsion troubles as an incidental benefit of using a salt - solvent pair has likewise been noted by other authors, *e.g.*, by Horning *et al.*[48] who added a saturating amount of ammonium carbonate to urine or plasma samples. An example of salt addition in the steroid field (neutral steroids) is the addition of ammonium sulphate solution at pH 1, prior to the extraction with ethyl acetate.[34] Extraction at an acidic pH has the attendant advantage of lessening the risk of emulsion formation.

An example of practical information in the literature concerns the extraction of plasma with 1,2-dichloroethane[49]; the organic phase sometimes formed a gelatinous solid emulsion. If its formation was not prevented by the layering procedure outlined above under Initial Treatment of Plasma Samples, an effective remedy was to stir the contents of the tube vigorously with a glass rod and then re-centrifuge them. Another example comes from our own laboratory; A. D. R. Harrison sometimes noted a haze, prejudicial to fluorimetry, in the alkaline, aqueous phase from the back-extraction of heptane extracts. This haze, attributable to heptane droplets, could be obviated by centrifuging at 5 °C rather than at room temperature. Similar haze problems following back-extraction from chloroform extracts can often be removed by bubbling nitrogen through the aqueous phase.

With some systems, mere standing without centrifugation rapidly brings about a clean separation of the phases, uncontaminated by denatured protein. Thus, centrifugation can be omitted when plasma is extracted with benzene,[9] chloroform[29] or a mixture of nonane and propan-2-ol, as in the assay of triglycerides. Such systems are essential for automatic or semi-automatic extractors, which lack provision for centrifugation.

Stockwell and Sawyer[50] (see also reference 51) remark: "Solvent extraction systems may be used to effect concentration, clean up or to provide both functions. There is no universally available automatic equipment which will combine the features required to carry out both functions on widely varying volumes of original sample." Semi-automatic apparatus, devised by Brown *et al.*[52] and somewhat resembling a counter-current distribution apparatus, is available commercially, but the fact that it is tailored to urinary oestrogen assays limits its versatility. This criticism is less applicable to another commercially available instrument (designed by P. F. Dixon), which is based on a sampler turntable with a stirrer and dipping

probes. To achieve accurate control in automatically removing a phase, an interface-sensing device (*e.g.*, reference 53) is needed, but this is seldom warranted in routine assays. It is still common practice, albeit a tedious one, to remove a portion of one phase manually with the aid of a bulb-controlled pipette. Hydrophobic phase-separating filter-paper can help,[54] although it may introduce contaminants such as tin.[55]

Automatic handling by the discrete approach is more likely to gain in popularity than is the continuous-flow approach, although the latter can be adapted (*e.g.*, reference 56) to handle two-phase systems.[50,51] Flow through a coil is also the key feature of an ingenious counter-current chromatographic apparatus that might justify the optimism of its originators in which a coil is continuously subjected to gentle centrifugation.[57]

Development of Solvent Methods

Certain aspects, including relative volumes, have been touched on in the sections on Ionisability and Other Chemical Features, Initial Treatment of Plasma Samples and Solvent Extraction (see also references 38 and 58). The so-called salt - solvent pair approach[59] allows even a water-miscible solvent such as ethanol to be used in small amount, although the efficiency may be poor. The choice of solvent may have to take account not only of safety and other aspects already considered, but also of any manipulative preference for a low or high density compared with water. With 1,2-dichloroethane a useful lowering of density may be achieved by admixing a non-polar solvent such as cyclohexane.[11]

Except with amphoteric compounds,[11] the pH is normally set so that the sample compound is un-ionised, unless it is to be back-extracted from an organic phase for the purpose of purification or of obtaining an aqueous solution for analysis. When a basic drug is unstable at an alkaline pH, a near-neutral pH may be suitable for its extraction into a solvent (*e.g.*, a mixture of chloroform and propan-2-ol) if an acidic agent, such as bromocresol purple, is added so as to yield a salt; this salt may conveniently dissociate when a thin-layer chromatogram is subsequently performed.[49,60] With urinary metadrenalines at an alkaline pH, Coward and Smith[61] found that addition of an aldehyde, such as dodecanal, with cyclohexanone as solvent, gave improved extraction, presumably as a result of complex formation; however, A. A. A. Aziz, in our laboratory, could not achieve efficient extraction thereby, and these authors[62] abandoned solvent extraction in favour of an ion-exchange approach.

Useful though it may be on occasion to choose an extractant that will interact chemically with the desired trace substance, solvent interactions occurring inadvertently can be troublesome. Examples of such interactions include imine formation from primary amines with acetone, oxazolidine formation from β-hydroxylated secondary amines with ketonic solvents and decomposition of some drug metabolites by ethyl acetate; interaction can also occur between halogenated hydrocarbons and many basic compounds.[44]

Chin and Fastlich[27] list a number of drugs that are extractable (in the presence of sodium dihydrogen orthophosphate) with diethyl ether but not with hexane. Ethyl methyl ketone at pH 2·0 was notably effective for quercetin.[41] The stratagem of adding a trace amount of an alcohol to a hydrocarbon extractant, mentioned in the section on adsorptive losses,[9] can also overcome problems of inefficient and variable extraction, as encountered with pronethalol and propranolol,[63] for which heptane containing 1 per cent. of ethanol was highly effective at pH 10·2. This stratagem has been successful not only with weakly basic drugs but also with weakly acidic drugs, such as atromid, where 2,2,4-trimethylpentane containing 5 per cent. of ethanol works well at acidic pH values; other mixtures that have been chosen empirically for various drugs include cyclohexane containing 2 per cent. of isoamyl alcohol (mainly 3-methylbutan-1-ol) or 5 per cent. of butan-1-ol, and heptane containing 1·5 per cent. of isoamyl alcohol (B. Scales, personal communication).

Consideration may have to be given to the question of selectivity.[12] The main variables that can be manipulated are pH and solvent polarity. For barbiturates in plasma, in the context of spectrophotometric determination, Bush and Sanders-Bush[64] (see also reference 9) write: "The least polar solvent that will give a reasonable recovery of drug will also extract minimal amounts of 'impurities' which often interfere in subsequent steps of an analysis."*

* In terms of polarity,[12] hydrocarbons and carbon tetrachloride can be grouped together, followed by a group that includes chloroform, diethyl ether and, at its upper end, 1,2-dichloroethane, and then by a group containing 1,4-dioxan, ethyl acetate and pentan-1-ol; the propanols have particularly high polarity, comparable with that of ethanol.

When the nature of interfering impurities is unclear, reliance has to be placed on an empirical approach. The following examples may be informative.

For the gas - liquid chromatographic determination of glutethimide and a more polar active metabolite, Hansen and Fischer[65] achieved efficient extraction by use of diethyl ether (or chloroform, but not light petroleum) at an acidic pH, but had to remove impurities by subsequent extraction into hexane from an ethanolic solution, with aqueous hydrochloric acid present so as to give two phases. The separation of fluphenazine from its metabolites can be achieved by an approach that entailed a quantitative study of extraction behaviour in relation to pH with a solvent of appropriate polarity.[66] For urinary 5-hydroxyindol-3-ylacetic acid, elaborate solvent-extraction steps to ensure specificity in the final colorimetry can be replaced by a simple ether extraction (with hydrochloric acid and sodium chloride present) if 2-mercaptoethanol is finally added to suppress any adventitious colour.[67]

Care in choosing the first extractant can reduce the need for subsequent clean-up extractions. In the assay of serum for quinidine, benzene extraction conveniently left irrelevant metabolites in the aqueous phase.[2,31] For various drugs in carbonate-saturated solutions, Horning *et al.*[48] found that propan-2-ol, although as efficient as ethyl acetate, extracted impurities that proved to be confusing in subsequent chromatography.

That butan-1-ol may be too powerful an extractant (and thus give high blanks) was demonstrated in a thorough study by Anton and Sayre,[43] who relied on carefully chosen solvent treatments in order to extract metadrenalines free from the parent catecholamines. It is illuminating to consider their elaborate series of extractions, each followed by centrifugation, as applied at a near-neutral pH to urine, or, after removing protein centrifugally at an acidic pH, to plasma or a tissue homogenate. (1) Add isoamyl alcohol in order to remove contaminants (this is used together with a judiciously chosen buffer); remove the aqueous layer free from interface pigment and then (2) adjust to pH 10·0 with borate solution and saturate with dipotassium hydrogen orthophosphate, added as a solid. (3) Add diethyl ether; save the ether layer and (4) use 0·1 M hydrochloric acid to back-extract. (5) Next repeat steps (2) and (3), then (6) back-extract into 0·01 M hydrochloric acid in order to obtain a solution for assay by differential fluorimetry. Over-all recoveries as high as 50 per cent. were reported for this technique, but Weil-Malherbe[68] had less success.

Step (1) above illustrates the not uncommon practice (*e.g.*, references 3 and 41) of pre-extracting so as to remove potentially interfering substances into an organic phase, which is then discarded. Thus, in order to assay metadrenalines Gupta *et al.*[69] pre-extracted with ethyl acetate (three times) at pH 1. In this method the subsequent extraction of metadrenalines from the aqueous phase (into toluene) is preceded by an oxidation step, in order to obtain an ultraviolet light absorbing product (vanillin), which is then back-extracted from toluene into carbonate solution before the spectrophotometric determination, the over-all outcome being high specificity and, it is claimed, quantitative recovery.

The extraction of reaction products, as in the foregoing example (see also reference 70), can be a very useful approach, when applicable. Thus, urine that has been subjected to treatment with borohydride and periodate can be extracted first with 2-methylbutane and then with ether to extract different derived 17-oxosteroids for determination of the 11-oxygenation index.[71] Quercetin can be converted into a fluorescent complex that is extractable into diisopropyl ether.[41] In the presence of a barbiturate and of calcium ions tetracyclines can form a fluorescent complex which, even if present in trace amount, is extractable into a solvent such as chloroform.[72] Dill *et al.*[19] have done notable work on the fluorescent dye method. In principle, it may be possible to achieve selectivity merely by chemical masking of unwanted compounds, *e.g.*, of primary amines when a secondary amine is to be assayed by derivative formation from isotopes.[70]

It is illuminating to read Glazko's description[73] of how a simplified method for the spectrophotometric determination of diphenylhydantoin (phenytoin) was developed. As a prelude to alkaline oxidation, in which chloroform would interfere, the drug (a weak acid) was extracted from plasma at pH 6·8 with 1,2-dichloroethane and then back-extracted into 1 N sodium hydroxide solution. Whereas other authors had steam-distilled the benzophenone formed by oxidation at 100 °C, Glazko collected it in an overlying layer of alumina-purified 2,2,4-trimethylpentane, which projected above a gasket so that it could be air-cooled without exposure to steam heat. Glazko[74] discusses the specificity of the method, its merits compared

with a gas - liquid chromatographic approach and the high sensitivity attainable by using fluorimetric measurement.

Separation with a Solid Additive or Column Material

To some extent, logic can guide the choice of a solid phase and of the correct working conditions to select a desired solute from aqueous solutions or remove unwanted solutes.[12] This process is usually carried out with a column, although batch operation is sometimes practised. However, there is an empirical element even when the work is in the hands of knowledgeable investigators. The terms adsorption, partition and ion exchange do not imply mutual exclusiveness in the separation mechanism. Whatever the solid phase, there is a risk that a small absolute amount of the solute may be retained, apparently irreversibly; the percentage loss can be serious when the solute is a trace constituent of a body fluid. Even with ion-exchange resins, there can be inadvertent absorptive losses, although as mentioned below, they can be minimised by adding an organic solvent or urea.

Alumina and Charcoal

Alumina represents a classical example of the use of an adsorbent, having long been recognised as an effective adsorbent for adrenaline or other aromatic compounds with vicinal hydroxyl groups when used at a pH in the region of 8·5.[14,75] Metadrenalines lack vicinal hydroxyl groups and should not be adsorbed; thus, when it is these metabolites rather than the parent catecholamines that are to be determined, alumina treatment may still be advantageous.[14,68] With catecholamines, Wong *et al.*[15] found that they had to keep the amount of alumina very small as, on following published procedures, recoveries as low as 10 per cent. were sometimes obtained; interference in gas - liquid chromatography due to impurities in the alumina was also observed.

One advantage possessed by alumina, and by adsorbents in general, is that comparatively high concentrations of salt, as found in urine, can be tolerated even when augmented as a result of acid hydrolysis followed by neutralisation.[14] Nevertheless, adsorbents have not come into widespread use in the present context of sample preparation. There may be lingering suspicions about batch-to-batch variability and, at least with urine, about between-sample variations, which might jeopardise the hope of adsorbing the desired constituent quantitatively with minimum adsorption of unwanted constituents. This hope is most likely to be realised if the adsorption isotherm is steep at low concentrations of the constituent. It is immaterial that it may flatten off at high concentrations that will never be encountered in practice.

The isotherms for adsorption of certain fatty acids on to charcoal (tested as a column) were shown by Hagdahl *et al.*[76] (working in Uppsala) to depend on the medium. Whereas there was a steep initial portion with 50 per cent. *m/V* ethanol as the medium, with 95 per cent. ethanol the graph was linear (and shallow for hexanoic and octanoic acids, although not for butyric acid). The isotherm for phenylalanine was slightly depressed, although unaltered in shape, when 0·1 M hydrochloric acid was used in place of water, but there remained the problem of achieving efficient elution. This led the Uppsala group[76] to investigate the use of charcoal that had been desaturated (deactivated) suitably by treatment with hexan-1-ol (0·1 per cent. in water). This technique gave lower isotherms, still non-linear for phenylalanine and tryptophan: "It is not certain until the isotherms have been very considerably depressed, that desorption equilibrium will be readily established, as adsorption is very strong at low concentrations (less than 10 mM)." Isotherms for various amino-acids were sufficiently different to allow chromatographic separation.

In order to adsorb urinary aromatic constituents, while not attempting to resolve them, Asatoor and Dalgliesh[77] used charcoal that had been deactivated with a long-chain aliphatic compound such as stearic acid and usually obtained efficient elution by use of water containing 5–10 per cent. *m/V* of phenol. For compounds with a basic (amino) group, these authors recommend that the elution be at an acidic pH, or that the deactivator be octadecylamine. In our own studies of urinary metadrenalines, which can be adsorbed at a neutral pH, A. A. A. Aziz has obtained good recoveries with stearic acid (in ethanol) as deactivator, but not with octadecylamine; acidified methanol was a good eluting agent, whereas aqueous phenol was not. Separation from urinary pigments was readily achieved. A. J. Winter has

noted that corticosterone and acidic compounds, in contrast to metadrenalines, are held rather tenaciously. Charcoal that has been stored exposed to air may lose its adsorptive efficiency, as observed in the pioneer studies in Uppsala,[78] which studies are informative concerning the properties and processing of charcoal.

Active charcoal was used to adsorb a urinary imipramine metabolite, which was then eluted with aqueous ethanol, apparently non-quantitatively.[79] This metabolite was shown to be a glucuronide. Meola and Vanko[80] describe a drug screening procedure that is based on adsorption of the drug from urine at pH 11 with Norit A charcoal (neutral, pharmaceutical grade), followed by elution with diethyl ether or a mixture of chloroform and propan-2-ol, but give no information on recoveries. However, in the drug assay field the literature on the use of charcoal is sparse, and hardly takes cognisance of the pre-1960 Uppsala literature cited above, or of potentially relevant radioimmunoassay literature, describing methods in which the carbon particles are pre-coated with a protein or other compound of high relative molecular mass and the question of elution does not arise. Here the aim is merely to remove unbound compound selectively after ligand treatment; with urine there may be poor adsorption owing to interference by urea and creatinine.[81]

Ion-exchange Resins

As the voluminous literature testifies,[14] urinary catecholamines and their metabolites have been a focus for the trial of cation-exchange resins in sample preparation. The resin treatment may be preceded by a procedure such as adsorption on to alumina, or the resin may be applied directly to a urine sample if the problem of interfering cations is circumvented. A simple procedure[82] which, in our experience, works better than more complex procedures, consists in passing a diluted urine sample at near-neutral pH, after acid hydrolysis, through a short Dowex 50 (ammonium) cation-exchange column, in the presence of urea so as to minimise adsorption. Metadrenalines are eluted with dilute ammonia solution and survive subsequent concentration, whereas catecholamines are destroyed (as we have confirmed). The de-salted concentrate of metadrenalines thereby obtained, although free from amino-acids, is still too impure for fluorimetric analysis. It is therefore subjected to cation-exchange chromatography with carboxymethylcellulose under carefully defined conditions, although this method is partly empirical,[82] or with cellulose phosphate, which is a more refined method in that the two metadrenalines can be separated,[83] as had previously been claimed for a carboxylic resin of methacrylate type.[84]

A Dowex 50 (sulphonic resin) step performed with more elaborate conditions than those outlined above[82,83] has been described with several variations, *e.g.*, the use of hydrochloric or formic acid to allow unwanted compounds, such as catecholamines, to be removed. Despite meticulous descriptions of resin preparation and running conditions, as in a procedure[84] in which amino-acids were largely removed by a sodium acetate washing step, few published procedures have been readily repeatable outside the originator's laboratory. This contention also holds for procedures in which a carboxylic rather than a sulphonic cation exchanger is advocated[14,85] for the initial treatment of the urine, one advantage of this refinement being the greater purity of the resin as purchased. Stratagems such as the use of borate to elute catecholamines as complexes[84] likewise may not be readily repeatable. Therefore, one should not be too sanguine in expecting published cation-exchange procedures to work in one's own hands without special care and should be alert to possible batch variations in the resin itself. Notwithstanding these reservations, the potential selectivity of ion-exchange resins[86] is a strong incentive to use them in sample preparation despite the tedium of pre-washing and of re-attaining equilibrium when the composition of the inflowing liquid is changed. With weak cation exchangers (such as Biorex 70) the risk of impaired loading due to urinary electrolytes is greater than with strong (sulphonic) cation-exchangers. Weil-Malherbe[68] therefore advocated an electrodialytic de-salting procedure, but later[14] he accepted this risk, stipulating merely that the urine sample must not be so large as to saturate the column with inorganic cations. While he questioned the usefulness of diluting the urine, this step does seem to be a useful precaution.[82,85]

Difficulties due to salts can, of course, arise not only with urine but also with resin eluates that are to be re-run in a second ion-exchange column, unless only volatilisable components have been used for elution. Chalmers and co-workers[87–89] use pyridinium acetate, removable by freeze-drying,[90] in separating acidic compounds from urine (which can be loaded directly)

by anion-exchange chromatography with DEAE - Sephadex. With polystyrene-based anion exchangers,[8] such as Dowex 1, some acidic compounds may decompose or be retained too strongly.[88] Nevertheless, anion exchangers such as Dowex 1 (which may conveniently remain wet if the inflow of eluting agent is inadvertently interrupted) deserve to be more widely used for the removal of unwanted acidic constituents.

Investigators using strong anion or cation exchangers for sample preparation might take cognisance of early literature[86] on the successful use of organic solvents as eluting agents, the solvents acting partly in a partition mode. One example of the use of aqueous - organic systems is the addition of 1,4-dioxan to minimise adsorptive retardation with organic acids.[91] Ethanol can improve the elution of dopamine and 5-hydroxytryptamine from Dowex 50 resin with dilute hydrochloric acid, good recoveries being obtained even with very low loads; aqueous methanol is a useful first eluting agent.[92] It may, however, be technically difficult to achieve a smooth, bubble-free transition from an aqueous to an organic system with an ion exchanger, or with the non-ionic resin XAD-2.

Amberlite XAD-2 Resin

This hydrophobic polystyrene resin (Rohm and Haas Co., Amberlite series) lacks ionisable groups and acts as a weak adsorbent; it is potentially valuable for removing trace constituents that possess a hydrophobic moiety and must be isolated from an aqueous medium, such as urine. Once adsorbed on to a column of the coarse, porous beads, the compound can be eluted with an agent such as methanol.

On isolating morphine (with a good recovery) and its metabolites from urine, Fujimoto and Wang[93] found that the methanol used to elute substances from the XAD-2 column first removed urinary pigments, which therefore served as a useful advance signal. Subsequent thin-layer chromatography gave a pattern which was much cleaner than that for material which had been solvent-extracted from hydrolysed urine. Mulé *et al.*[94] examined the behaviour of XAD-2 resin towards urine that was spiked with various drugs of abuse. The eluting agent selected was chloroform - propan-2-ol. Whereas some drugs, such as phenobarbital, were efficiently adsorbed and eluted, with others there was poor adsorption and/or elution. With morphine (recovery only 64 per cent.) and especially with its glucuronide (recovery nil) the main difficulty was incomplete adsorption, which was not remedied by adjusting the pH to 9.

Kullberg and Gorodetzky[32] treated urine at pH 8·5 with XAD-2 and eluted with methanol - chloroform, which gave a clean extract for subsequent thin-layer chromatography. They obtained 60–80 per cent. recovery for morphine and various other drugs of abuse, except for aspirin (which was poorly adsorbed). They describe important points of technique (see also references 95 and 96). Elution with organic solvents entails technical difficulties that are associated partly with the displacement of residual water from the column, and can be minimised by prior passage of a small volume of acetone. This water may form, in the collecting tube, an upper phase which, advantageously to thin-layer chromatography, contains unwanted urinary pigments but which would also contain much of the eluted drug if it was not deliberately saturated with ammonium chloride solution at pH 10 (see also reference 94).

Adsorptive resins, such as XAD-2, are evidently not a panacea for sample preparation. XAD-2 scarcely adsorbs purine analogues (A. Bye, personal communication), and in our laboratory it has not consistently given a good recovery of both metadrenalines. Its efficacy may vary with different urines, perhaps reflecting erratic selectivity for unwanted urinary constituents in addition to the compound under study.[96]

XAD-2 resin is not normally used to separate one drug from another, although potentially it could achieve this.[54,97] Moreover, the efficacy of XAD-2 in dealing with morphine glucuronide is controversial.[32,93,94] The stratagem of hydrolysing the glucuronide on the XAD-2 column gave only a 40 per cent. over-all recovery, although the thin-layer chromatographic patterns were cleaner than for material separated by XAD-2 from hydrolysed urine.[32] However, good recovery of a sulphate conjugate by using XAD-2 has been reported in the isolation of bile acids from urine.[98]

Other Agents

For aromatic constituents of urine (not quantified), Vallon *et al.*[99] used Sephadex G-10 in an adsorption mode at pH 1, and on raising the pH they achieved selective elution, apparently

less facile with conjugates. Oestrogen conjugates can be isolated, perhaps with some inadvertent selectivity, by the aid of Sephadex.[100,101] Chromatography on lipophilic Sephadex[102] with organic solvents has served as an intermediate step in cleaning up extracted material for thin-layer chromatography. Thus Makino *et al.*[98] (*cf.* reference 102) used Sephadex LH-20 to chromatograph a chloroform - methanol solution of sulphated and non-sulphated bile acids that had been isolated from plasma or urine by XAD-2. This chromatography, which in the case of the sulphate fraction was followed by hydrolysis, gave bile acids, which in turn were converted into methyl esters and purified in benzene solution on an aluminium oxide column. Another study in the steroid field has entailed the use of Sephadex rendered rich in hydroxyalkoxypropyl residues.[103,104] In the isolation of homovanillic acid from rat urine, lipophilic Sephadex facilitated the final clean-up (unnecessary with human urine) after chromatography on silicic acid of a solution in chloroform.[105]

Dry silica gel, when added to acidified urine, selectively extracts weak carboxylic acids, which can then be eluted from the gel (as a column) by organic solvents.[106] Silica gel can also adsorb from a dichloromethane extract.[107] For the removal of coloured impurities, an aqueous solution of metadrenalines isolated from urine for fluorimetric measurement was treated with Florex (an aluminium silicate) in the presence of urea[82]; significant losses were later encountered, however.[83]

Choice of Approach

In addition to the various pre-separation procedures surveyed above, there remains the option of chromatography on a thin layer, as applied to chlorpropamide and its metabolites,[108] or on suitably pre-treated[109] paper. Chromatography on a conventional film or sheet may, however, be inappropriate because only a low load of the salt-free urine or other test fluid can be applied, even if streaked rather than spotted. This possible limitation also applies to sheet electrophoresis, as used with pre-concentrated urine for metabolites of ^{3}H-noradrenaline, in which the metabolites are separated on paper in a borate buffer at 300 V.[110] Another conceivable approach, hardly justifiable in practice, is preparative gas - liquid chromatography.

A possible variant of the solvent approach, carried out in the interests of efficiency if not selectivity, is liquid - solid extraction after freeze-drying the sample.[38,111] Freeze-drying under carefully chosen conditions has proved to be a useful intermediate step in the analysis of urine for organic acids,[87] but seems unlikely to be widely adopted.

If the final analytical step entails chromatography with a small loading volume, the sample preparation approach must be appropriate for obtaining the sample in a concentrated form. Semi-purified samples may have to be evaporated, taking all necessary precautions; thus, an organic base may have to be dried as its hydrochloride in order to obviate volatilisation. Even when solvent removal can be carried out at atmospheric pressure, suitably with the aid of a manifold and a stream of nitrogen,[112] it is a tedious operation. However, the need to use only a minute volume of solvent for meticulously dissolving the residue can be obviated, when a chromatographic plate has to be loaded, if an automatic device for repeated spotting is available, thus allowing the use of a larger volume.[113] As already mentioned in connection with ion-exchange chromatography, difficulty arises if involatile salts are present. This problem does not apply if solvent-extraction procedures have been used.

Generalisations and Examples

Solvent extraction is generally the ideal method if, as happens all too rarely, a single primary extraction (followed, if necessary, by one washing step and a back-extraction) provides a good recovery (in terms of amount and condition) of the desired compound. Conditions can be manipulated to give selectivity of extraction (see the section on Development of Solvent Methods). Clifford and Smyth[35] give an illuminating survey in the context of 1,4-benzodiazepines with different pK values and note that diethyl ether at pH 7–9 is often an effective solvent. The statement that amphoteric or neutral compounds do not lend themselves to solvent extraction[58] is rather sweeping. In the toxicological field, alternative approaches such as chromatography were still rare when the applicability of classical chromatographic methods was reviewed by Kirk[13] in 1968. (HPLC has only recently achieved recognition, as is discussed below; it is hardly a ready means of sample preparation.)

Among the few direct comparisons of solvent extraction with alternative procedures is the report by Miller *et al.*,[96] whose XAD-2 results with urinary morphine (see also reference 32) were "comparable to or better than those obtained by liquid - liquid extraction techniques. In addition, the studies show that morphine extracted from urine by XAD-2 resin can be stored on the resin for 14 d without degradation." In his survey of methods for determining urinary catecholamines and related compounds, Weil-Malherbe[14] emphasises the need for a selective solid-phase step (adsorptive or ion-exchange), and sees no advantage in having a prior solvent-extraction step. Indeed, as indicated in the section on Development of Solvent Methods (see also reference 62), metadrenalines are not readily obtained in good yield and purity by solvent extraction. Because, however, adsorption on alumina does not itself guarantee specific fluorescence measurements in the instance of catecholamines from acid-hydrolysed urine, the eluate from the alumina column should be put through an ion-exchange step.[68]

Ion-exchange column procedures, while being tedious and sometimes difficult, are evidently advantageous for discriminatory separations. When partition coefficients are unfavourable for solvent extraction (as with some organic acids[88]) or when the eluate can be assayed directly by means of a photometric procedure, the ion-exchange approach is particularly attractive. The narrower the column, the slower will be the loading step but the better will be the prospect of eluting the desired constituent in a small volume. In order to obtain good resolution it may be helpful to use a long column, to use unorthodox eluting agents (*e.g.*, reference 92) and also to use a gradient (smooth or stepped), a stratagem that has understandably not found favour in connection with body-fluid assays. Poor recoveries with low loads (see section on Ion-exchange Resins) are a particular hazard, which may go undetected if model experiments are performed only with loads of the trace substance that are unrealistically high.

Once the conditions have been optimised for a chromatographic separation, ion-exchange or otherwise, it should be possible to dispense with a fraction collector and to collect standardised portions of eluate, particularly if the separation does not have to be rigorous and a short column suffices. It may even be feasible to operate in a batch mode rather than with a column, or to use ion-exchange paper (see reference 32). However, so-called liquid ion exchange (as used in a steroid study[114]) is really a refinement of the solvent-extraction approach that depends on ion pairing; use of the term has been strongly condemned.[12] Ion pairing is in fact a powerful aid to efficiency and discrimination both in partition chromatography and in solvent extraction.[115]

Notable difficulties in choosing conditions for sample preparation are exemplified by the literature in two fields. The steroid field abounds with examples of multi-stage techniques,[109] *e.g.*, the successive use of solvent extraction, ion-exchange and thin-layer chromatography for urinary oestrogens in trace amounts as a prelude to their determination (as esters) by gas - liquid chromatography.[116] Adlercreutz[101] considers that, for pregnancy oestrogens, sample preparation needs to be more rigorous with gas - liquid chromatography than with colorimetry or fluorimetry. The biogenic amine field has likewise proved to be a test of patience and judgement: "values (for metadrenalines) obtained from 24-h samples of urine collected from normal individuals vary greatly according to the method used, even to the point where values analyzed in the same laboratory at different times seem to differ."[82] Procedures entailing solvent extraction, ion exchange or adsorption on alumina have already been discussed. When the two metadrenalines have to be measured individually in the same sample, the risks of differential determination by a non-separatory method, such as fluorimetry, may have to be accepted. Preferably, however, the compounds should be separated, and this can be achieved for metadrenalines by use of cellulose phosphate chromatography[83] or by gas - liquid chromatography after derivative formation. Thin-layer chromatography has also been used, for the challenging problem of determining adrenaline and noradrenaline in plasma.[117] Following the enzymic introduction of radioisotopically labelled methyl groups, the pair of metadrenalines were selectively solvent extracted, with rather low efficiency, before the thin-layer chromatographic separation, after which each was eluted and oxidised to vanillin, which was counted following solvent-extraction steps.

The efficacy of gas - liquid chromatography in drug analysis has been well reviewed by Riedmann,[36] who considered the sample preparation aspect and the derivative-formation step that is usually unavoidable. If a nitrogen-selective detector is used, a simplified sample preparation procedure may suffice.[118] Whenever sample preparation demands a sequence of

steps, these should depend on different properties of the compound(s) to be separated or removed as one of the principles that should govern any complex separation.[12] Options that have been considered above are summarised in Fig. 1.

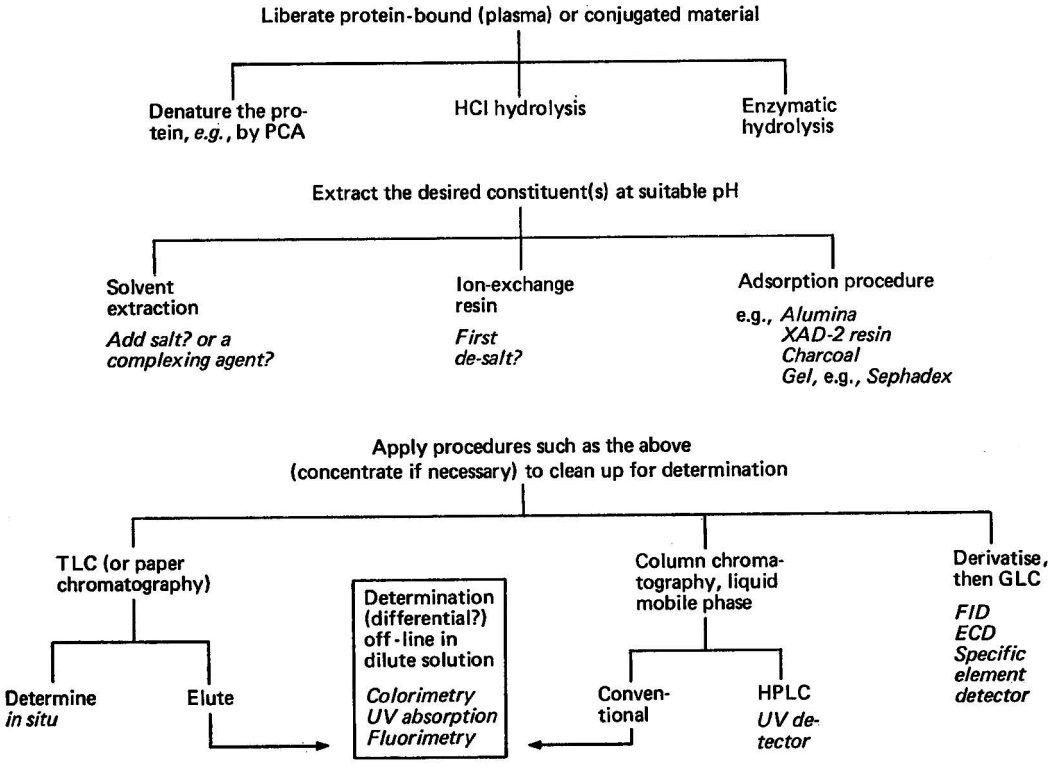

Fig. 1. Possible stages in the assay of plasma or urine for organic compounds of low relative molecular mass (*e.g.*, drugs or catecholamine metabolites) present in trace amounts.

Trends in Methods of Sample Preparation

Difficult though it is to generalise, some salient points can now be stated.

(1). In connection with bioavailability assessment and therapeutic monitoring, there is an increasing need to obtain reliable values (say within ± 5 per cent.) for drug levels, particularly in plasma, at concentrations that may be below 100 $\mu g\ l^{-1}$.

(2). When present-day assay methods purport to achieve the above, with some assurance of specificity, successful operation may call for experience, dexterity and patience, and may be costly in terms of man-hours.

(3). In order to achieve the requisite sensitivity and discrimination by use of approaches other than radioimmunoassay or related affinity methods, an adequate sample-preparation procedure (Fig. 1) must be used, which takes account of any need to measure conjugates (see section on Methods of Handling Conjugates, below) or other metabolites.

(4). This procedure may have to be rather elaborate [*cf.* (2), and section on Concluding Comments, below], possibly in the interests of obtaining low blanks as well as good discrimination when there is a classical photometric end-measurement (colorimetry, fluorimetry or ultraviolet absorptiometry; Fig. 1) that is not concomitant with a chromatographic separation.

(5). For such a separatory measurement it has become traditional to use gas - liquid chromatography. While improved techniques for forming derivatives and detection are helping to keep gas - liquid chromatography in the forefront of methods, its protagonists need to be alert to two alternative approaches that do not entail derivative formation. One is thin-layer chromatography, which can readily give tolerably good precision and productivity

now that automatic instruments for applying spots and for scanning them *in situ* have become available.[113] The other is high-pressure liquid chromatography.

The Advent of High-pressure Liquid Chromatography (HPLC)

When Michaelis *et al.*[119] reviewed this powerful technique and its pharmaceutical applications in 1973, many trace constituents were already amenable to analysis by HPLC. Even disregarding mere model separations of unproved applicability to plasma and urine, the list has become increasingly impressive.[120] However, in a recent HPLC study of phenothiazine metabolites that had been isolated from urine by ion-exchange and solvent-extraction procedures, Landgraf[121] was not greatly impressed by his HPLC results, particularly the quantitative aspects (depending on measurements made at a wavelength of 254 nm). This contrasts with the enthusiasm of the Oak Ridge group, who have been ambitiously applying HPLC (not really high speed) to "profile" whole urine in the context of screening.[122] Recently[25] they have applied anion-exchange HPLC to a serum or urine ultrafiltrate as a means of following the metabolic fate of acetaminophen, when given in high dosage. HPLC with a pellicular cation exchanger has been employed to follow the fate of ingested furosemide[16]; with unprocessed serum or urine a concentration of the order of 5 mg l^{-1} could be measured readily.[123]

When comparatively high concentrations are being analysed, it may be valid to use raw samples, preferably protecting the column with a disposable pre-column. In general, however, it is advisable to apply sample-preparation steps initially.[108,124] In scanning urine by HPLC, which otherwise would be performed directly on the urine, batchwise pre-treatment with a cation exchanger served to reduce noise due to ultraviolet-absorbing basic constituents of urine.[125] In attempting to carry out the determination by cation-exchange HPLC of urinary metadrenalines at concentrations below 100 μg l^{-1}, J. P. Leppard, in this laboratory, found it essential to reduce noise by a sample-preparation step such as treatment with Dowex 50.[82] In determining theophylline, Manion *et al.*[54] found that elimination of caffeine interference from plasma (by solvent extraction) was less important with HPLC than with gas - liquid chromatography; an XAD-2 column step had to be adopted in the HPLC analysis of urine. Usually, the sample-preparation procedure need not be elaborate as HPLC allows good discrimination, if not sensitivity.

HPLC is, unfortunately, less sensitive than gas - liquid chromatography because of the present-day reliance on ultraviolet detection. Fluorimetric detection may gain in popularity when a reagent-addition facility becomes a standard feature of HPLC apparatus, enabling fluorescent derivatives to be formed after the separation.[120] Electrochemical detection is a promising technique but is not without difficulties, among which is the constraint of having to use acidic, de-gassed eluting agents. Already, it has, in conjunction with a cation exchanger, enabled L-dopa to be determined in serum at a level of 100 μg l^{-1}, following deproteinisation (with perchloric acid) and an alumina column step, which achieved a 20-fold concentration.[126] Even this level of sensitivity, as obtained by use of a concentrate, is far less impressive than that of gas - liquid chromatographic analysis. Chemical ionisation mass spectrometry used on-line with HPLC could give good sensitivity, perhaps with some sacrifice of HPLC resolution. This mass-spectrometric approach has even been applied directly to drugs and metabolites in plasma, with no sample preparation other than extraction into benzene; deuterated derivatives of the compounds were added as internal standards.[127]

Field desorption mass spectrometry is, in principle, well suited to HPLC eluates, but it is rather insensitive; it could, however, be advantageous in enabling intact conjugates to be examined without chromatographic separation.[128]

Methods of Handling Conjugates

Although conjugates are rather involatile for gas - liquid chromatography, they may be amenable to analysis by HPLC. The above HPLC analysis of urine after ingestion of acetaminophen[25] gave sulphate conjugates late in the elution process (near 21 h). In a preliminary report,[129] which, however, hardly inspires confidence, the glucuronide and the sulphate of *p*-hydroxyacetanilide, of which the ingested dose was high, gave good HPLC peaks on an anion exchanger with a 1-μl urine sample, but other sulphates were difficult to elute. Before the advent of HPLC, conventional ion-exchange columns were shown to be

capable of handling sulphate conjugates of urinary metadrenalines (at acidic pH) and[84,130] glucuronide conjugates of dopamine metabolites.[131]

Evidently, then, initial hydrolysis is not obligatory when urinary conjugates have to be determined. For sample preparation organic solvents are likely to be ineffective[88] unless the extraction is effected at pH 1 (inapplicable to basic drugs)[48,103,104] or an ion-association system[12,114,115] can be found. Isolated observations cited in the section on Separation with a Solid Additive or Column Material indicate that charcoal, Sephadex and XAD-2 may sometimes be effective in extracting conjugates.

It is sometimes of intrinsic interest to ascertain what proportion of a metabolisable compound is converted into conjugates *in vivo*.[103,130] Usually, however, the advantage of extracting intact conjugates lies rather in tidier analyses, insofar as initial hydrolysis of urine may sometimes create problems later (see Hydrolysis of Conjugates). Acid hydrolysis is particularly likely to create noise problems when the conjugate needs rigorous conditions to ensure complete hydrolysis, as exemplified by the sulphates of certain steroids including bile acids. Such conditions may be analytically innocuous if applied to semi-purified conjugates, rather than to the original urine, as in a study[98] cited under Other Agents. In this study, bile acid sulphates were split solvolytically by standing them for 2 d in ethanol - acetone at pH 1, then neutralising and drying them; the residue was heated for 4 h at 120 °C in 15 per cent. sodium hydroxide solution. In an infant steroid investigation,[103,104] incubation of an ethyl acetate extract obtained from urine at pH 1 led to solvolysis of sulphates but not of glucuronides. In the metadrenalines field, Fecher *et al.*[110] had some success with mild procedures for acid hydrolysis in the presence of an organic solvent.

Evidently, techniques are potentially available for handling intact conjugates, to be hydrolysed either unconventionally (not at the outset) or not at all. The feasibility of hydrolysing conjugates while they are adsorbed on XAD-2 or charcoal deserves investigation, in spite of one disappointing result.[32]

Concluding Comments

The next few years are likely to see piecemeal advances rather than major innovations in assay techniques for plasma and urine. HPLC is now being absorbed into the repertoire of approaches and will be rendered increasingly useful by advances in detector instrumentation[120] (see the section on HPLC above) and in separation systems. Ion-pair partition systems, as recently tried for biogenic amines and acidic metabolites,[132] offer selectivity in solvent extraction[115] as well as in chromatography.[133] Such a system has enabled extracted nucleotides to be freed from trichloroacetic or perchloric acid.[134]

Advances in sample preparation, relevant even to HPLC, will come largely from the skilful use of solid phases for isolative steps that are not readily effected with solvents. As indicated in the sections on Alumina and Charcoal and Amberlite XAD-2 Resin, even a simple adsorptive procedure may be convenient for concentrating or cleaning up, perhaps with modest selectivity. With ion exchangers, which are especially useful for basic compounds, judicious choice of conditions, particularly pH, can give selectivity in the loading step as well as in elution (*e.g.*, references 82 and 131). Ion-exchange membrane filters[135] warrant investigation for their possible usefulness in the present context of organic trace analysis.

There is a particular need for adsorptive procedures that will effectively extract compounds from plasma even if strongly protein-bound. This has already been achieved with thyroxine by use of Sephadex G-25 in a strongly alkaline medium.[136] A useful corollary would be ways in which to determine free compounds and protein-bound compounds separately.[137] Radioimmunoassay literature is relevant to the choice of adsorptive step, although not to the requisite desorption. Neglect to check how bond compounds behave is common.

Conventional sample-preparation procedures that have to be run repetitively warrant automatic, or at least work-simplified, approaches.[51,113] Here the cost-effectiveness aspect has to be considered, as it should be also in comparing different ways of assaying a particular compound. Account has to be taken of operator time, instrument cost and work-load, resolution attainable in the final measurement step and the desired precision and specificity.[5]

Bush[109] gives a warning about possible tribulations that could be encountered with unconventional methods, such as double isotope dilution. The setting up of a radioimmunoassay or other affinity method should not be lightly embarked on. Cost effectiveness may rule out the use, in multi-sample routine work, of lengthy methods with a low throughput,

good though they may be in respect of specificity. With drugs,[73] as with metadrenalines,[82,83] it may be worth having available both a routine method, possibly not very specific even when only a single drug has been administered, and a more elaborate method that meets the rigorous requirements of research investigations. Thus, anticonvulsants to be gas chromatographed can be isolated and methylated by a simplified procedure that obviates solvent evaporation.[138]

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References

1. Bassett, R. G., Wright, J. A., and Cravey, R. H., *Clin. Chem.*, 1975, **21**, 44.
2. Cramér, G., and Isaksson, B., *Scand. J. Clin. Lab. Invest.*, 1963, **15**, 553.
3. Moss, G. F., Jones, K. M., Ritchie, J. T., and Cox, J. S. G., *Toxic. Appl. Pharmac.*, 1971, **20**, 147.
4. Mulé, S. J., Bastos, M. L., and Jukofsky, D., *Clin. Chem.*, 1974, **20**, 243.
5. Butler, V. P., *Metabolism*, 1973, **22**, 1145.
6. Teale, J. D., Forman, E. J., King, L. J., and Marks, V., *Proc. Soc. Analyt. Chem.*, 1974, **11**, 219.
7. Murphy, B. P., and Pattee, C. J., *J. Clin. Endocr. Metab.*, 1964, **24**, 187.
8. Murphy, B. P., *J. Lab. Clin. Med.*, 1965, **66**, 161.
9. Brodie, B. B., Udenfriend, S., and Baer, J. E., *J. Biol. Chem.*, 1947, **168**, 299.
10. Titus, E. O., *in* La Du, B. N., Mandel, H. G., and Way, E. L., *Editors*, "Fundamentals of Drug Metabolism and Drug Disposition," Williams and Wilkins, Baltimore, U.S.A., 1972, p. 419.
11. Trevor, A., Rowland, M., and Way, E. L., *in* La Du, B. N., Mandel, H. G., and Way, E. L., *Editors*, "Fundamentals of Drug Metabolism and Drug Disposition," Williams and Wilkins, Baltimore, U.S.A., 1972, p. 369.
12. Karger, B. L., Snyder, L. R., and Horvath, C., "An Introduction to Separation Science," John Wiley and Sons, New York, 1973.
13. Kirk, P. L., *Adv. Chromat.*, 1968, **5**, 79.
14. Weil-Malherbe, H., *in* Glick, D., *Editor*, "Methods of Biochemical Analysis," Supplementary volume, "Analysis of Biogenic Amines and their Related Enzymes," Interscience Publishers, New York, 1971, p. 119.
15. Wong, K. P., Ruthven, C. R. J., and Sandler, M., *Clinica Chim. Acta*, 1973, **47**, 215.
16. Forrey, A. W., Kimpel, B., Blair, A. D., and Cutler, R. E., *Clin. Chem.*, 1974, **20**, 152.
17. O'Brien, J., Zazulak, W., Abbey, V., and Hinsvark, O., *J. Chromat. Sci.*, 1972, **10**, 336.
18. Brodie, B. B., Udenfriend, S., Dill, W., and Downing, G., *J. Biol. Chem.*, 1947, **168**, 311.
19. Dill, W. A., Chucot, L., Chang, T., and Glazko, A. J., *Anesthesiology*, 1971, **34**, 73.
20. Spirtes, M. A., *Clin. Chem.*, 1972, **18**, 317.
21. Wigfield, D. C., *Analyt. Biochem.*, 1975, **63**, 286.
22. Kushinsky, S., and Anderson, M., *Clin. Chem.*, 1974, **20**, 1528.
23. Brown, H. H., Vanko, M., and Meola, J. M., *Clin. Chem.*, 1974, **20**, 919.
24. Deome, A. J., *Clin. Chem.*, 1974, **20**, 1383.
25. Mrochek, J. E., Katz, S., Christie, W. H., and Dinsmore, S. R., *Clin. Chem.*, 1974, **20**, 1086.
26. Matin, S. B., Karam, J. H., and Forsham, P. H., *Analyt. Chem.*, 1975, **47**, 545.
27. Chin, D., and Fastlich, E., *Clin. Chem.*, 1974, **20**, 1382.
28. Carmichael, R. H., *Clin. Chem.*, 1959, **5**, 597.
29. Gardner-Thorpe, C., Parsonage, M. J., and Toothill, C., *Clinica Chim. Acta*, 1971, **35**, 39.
30. Goldberg, V. D., Rosewarne, C., and Lascelles, P. T., *Proc. Soc. Analyt. Chem.*, 1974, **11**, 288.
31. Härtel, G., and Harjanne, A., *Clinica Chim. Acta*, 1969, **23**, 289.
32. Kullberg, M. P., and Gorodetzky, C. W., *Clin. Chem.*, 1974, **20**, 177.
33. Anton, A. H., Serrano, A., Tjandramaga, T. B., and Goldberg, L. I., *Science, N.Y.*, 1973, **182**, 60.
34. Shackleton, C. H. L., Charro-Salgado, A. L., and Mitchell, F. L., *Clinica Chim. Acta*, 1968, **21**, 105.
35. Clifford, J. M., and Smyth, W. F., *Analyst*, 1974, **99**, 241.
36. Riedmann, M., *Xenobiotica*, 1973, **3**, 411.
37. Daley, R. D., *in* Florey, K., *Editor*, "Analytical Profiles of Drug Substances," Volume 1, Academic Press, New York, 1972, p. 119.
38. Jones, C. R., *in* Reid, E., *Editor*, "Methodological Developments in Biochemistry. 5. Assay of Drugs and Other Trace Organics in Biological Fluids," ASP Biological and Medical Press B.V., Amsterdam, 1976, in the press.
39. Farrand, R. J., *J. Clin. Path.*, 1966, **19**, 92.
40. Dill, W. A., and Glazko, A. J., *Clin. Chem.*, 1972, **18**, 675.
41. Gugler, R., and Dengler, H. J., *Clin. Chem.*, 1973, **19**, 36.
42. Sammons, H. G., Almond, S. J., and Botterill, V. M., *Ann .Clin. Biochem.*, 1969, **6**, 169.
43. Anton, A. H., and Sayre, D. F., *J. Pharmac. Exp. Ther.*, 1966, **153**, 15.
44. Beckett, A. H., *in* Porter, R., and O'Connor, M., *Editors*, "The Poisoned Patient," Ciba Foundation Symposium No. 26, Elsevier Publishing Co., Amsterdam, 1974, p. 57.
45. Jackson, J. V., *in* Clarke, E. G. C., *Editor*, "Isolation and Identification of Drugs," Pharmaceutical Press, London, 1969, p. 16.

46. Ramsey, J., and Campbell, D. B., *J. Chromat.*, 1971, **63**, 303.
47. Aggarwal, V., Bath, R., and Sunshine, I., *Clin. Chem.*, 1974, **20**, 307.
48. Horning, M. G., Gregory, P., Nowlin, J., Stafford, M., Lertratanangkoon, K., Butler, C., Stillwell, W. G., and Hill, R. M., *Clin. Chem.*, 1974, **20**, 282.
49. Brodie, B. B., and Udenfriend, S., *J. Biol. Chem.*, 1945, **158**, 705.
50. Stockwell, P. B., and Sawyer, R., *Lab. Equip. Dig.*, 1972, **10** (11), 51.
51. Foreman, J. K. F., and Stockwell, P. B., "Automatic Chemical Analysis," Ellis Horwood, Chichester, 1974.
52. Brown, J. B., MacLeod, S. C., Macnaughtan, C., Smith, M. A., and Smyth, B., *J. Endocr.*, 1968, **42**, 5.
53. Porter, D. G., Jackson, C. J., and Bunting, W., *Lab. Pract.*, 1974, **23**, 111.
54. Manion, C. V., Shoeman, D. W., and Azarnoff, D. L., *J. Chromat.*, 1974, **101**, 169.
55. Meakin, J. C., *Clin. Chem.*, 1973, **19**, 141.
56. Blackmore, D. J., Curry, A. S., Hayes, T. S., and Rutter, E. R., *Clin. Chem.*, 1971, **17**, 896.
57. Ito, Y., Hurst, R. E., Bowman, R. L., and Achter, E. K., *Separ. Purif. Methods*, 1974, **3**, 117.
58. Blanke, R. V., *in* Tietz, N. W., *Editor*, "Fundamentals of Clinical Chemistry," Saunders Publishers, Philadelphia Pa., 1970, p. 833.
59. Bastos, M. L., Kananen, G. E., Young, R. M., Monforte, J. R., and Sunshine, I., *Clin. Chem.*, 1970, **16**, 931.
60. Stoner, R. E., and Parker, C., *Clin. Chem.*, 1974, **20**, 309.
61. Coward, R. F., and Smith, P., *Chemy Ind.*, 1966, 210.
62. Coward, R. F., and Smith, P., *Clinica Chim. Acta*, 1966, **14**, 672.
63. Black, J. W., Duncan, W. A. M., and Shanks, R. G., *Brit. J. Pharmac.*, 1965, **25**, 577.
64. Bush, M. J., and Sanders-Bush, E., *in* Woodbury, D. M., Perwy, J. K., and Schmidt, R. P., *Editors*, "Antiepileptic Drugs," Raven Press, New York, 1972, p. 293.
65. Hansen, A. R., and Fischer, L. J., *Clin. Chem.*, 1974, **20**, 236.
66. Whelpton, R., and Curry, S. H., *in* Reid, E., *Editor*, "Methodological Developments in Biochemistry. 5. Assay of Drugs and Other Trace Organics in Biological Fluids," ASP Biological and Medical Press B.V., Amsterdam, 1976, in the press.
67. Goldenberg, H., *Clin. Chem.*, 1973, **19**, 38.
68. Weil-Malherbe, H., *Meth. Biochem. Analysis*, 1968, **16**, 293.
69. Gupta, R. N., Price, D., and Keane, P. M., *Clin. Chem.*, 1973, **19**, 611.
70. Chamberlain, J., *in* Reid, E., *Editor*, "Methodological Developments in Biochemistry. 5. Assay of Drugs and Other Trace Organics in Biological Fluids," ASP Biological and Medical Press B.V., Amsterdam, 1976, in the press.
71. Few, J. D., *J. Endocr.*, 1968, **41**, 213.
72. Kohn, K. W., *Analyt. Chem.*, 1961, **33**, 862.
73. Glazko, A. J., *in* Woodbury, D. M., Penry, J. K., and Schmidt, R. P., *Editors*, "Antiepileptic Drugs," Raven Press, New York, 1972, p. 103.
74. Glazko, A. J., *Clin. Chem.*, 1974, **20**, 915.
75. Drell, W., *Analyt. Biochem.*, 1970, **34**, 142.
76. Hagdahl, L., Williams, J. P., and Tiselius, A., *Ark. Kemi*, 1951, **4**, 193.
77. Asatoor, A., and Dalgliesh, C. E., *J. Chem. Soc.*, 1956, 2291.
78. Claesson, S., *Ark. Kemi Miner. Geol.*, 1946, **23A** (1), 1.
79. Herrman, B., and Pulver, R., *Archs. Int. Pharmacodyn. Thér.*, 1960, **126**, 454.
80. Meola, J. M., and Vanko, M., *Clin. Chem.*, 1974, **20**, 184.
81. Mortensen, E., *Clin. Chem.*, 1974, **20**, 1146.
82. Kahane, Z., and Vestergaard, P., *J. Lab. Clin. Med.*, 1967, **70**, 333.
83. Kahane, Z., and Vestergaard, P., *Clinica Chim. Acta*, 1969, **25**, 453.
84. Taniguchi, K., Kakimoto, Y., and Armstrong, M. D., *J. Lab. Clin. Med.*, 1964, **64**, 469.
85. Sandhu, R. S., and Freed, R. M., *Stand. Meth. Clin. Chem.*, 1972, **7**, 231.
86. Morris, C. J. O. R., and Morris, P., "Separation Methods in Biochemistry," First edition, Pitman and Sons, London, 1963.
87. Chalmers, R. A., and Watts, R. W. E., *Analyst*, 1972, **97**, 958.
88. Chalmers, R. A., *in* Reid, E., *Editor*, "Methodological Developments in Biochemistry. 5. Assay of Drugs and Other Trace Organics in Biological Fluids," ASP Biological and Medical Press B.V., Amsterdam, 1976, in the press.
89. Chalmers, R. A., and Lawson, A. M., *Chemy Britain*, 1975, **11**, 290.
90. Chalmers, R. A., Lawson, A. M., and Watts, R. W. E., unpublished work.
91. Davies, C. W., and Owen, B. D. R., *J. Chem. Soc.*, 1956, 1681.
92. Atack, C. V., and Magnusson, T., *J. Pharm. Pharmac.*, 1970, **22**, 625.
93. Fujimoto, J. M., and Wang, R. I. H., *Toxic. Appl. Pharmac.*, 1970, **16**, 186.
94. Mulé, S. J., Bastos, M. L., Jukofsky, D., and Saffer, E., *J. Chromat.*, 1971, **63**, 289.
95. Hetland, L. B., Knowlton, D. A., and Couri, D., *Clinica Chim. Acta*, 1972, **36**, 473.
96. Miller, W. L., Kullberg, M. P., Banning, M. E., Brown, L. D., and Doctor, B. P., *Biochem. Med.*, 1973, **7**, 145.
97. Chu, C.-H., and Pietrzyk, D. J., *Analyt. Chem.*, 1974, **46**, 330.
98. Makino, I., Shinozaki, K., Nakagawa, S., and Mashimo, K., *J. Lipid Res.*, 1974, **15**, 132.
99. Vallon, J. J., Badinand, A., and Bichon, C., *Clinica Chim. Acta*, 1972, **36**, 397.
100. Beling, C. J., *Adv. Obstet. Gynecol.*, 1967, **1**, 88.
101. Adlercreutz, H., *Clinica Chim. Acta*, 1971, **34**, 231.
102. Sjövall, F., Nyström, E., and Haahti, E., *Adv. Chromat.*, 1968, **6**, 119.

103. Anderson, R. A., Chambaz, E. M., Defaye, G., Madani, C., Baillie, T. A., and Brooks, C. J. W., *J. Chromat. Sci.*, **1974**, **12**, 636.
104. Anderson, R. A., Chambaz, E. M., and Brooks, C. J. W., *J. Chromat.*, **1974**, **99**, 485.
105. Dailey, J. W., and Änggård, E., *Biochem. Pharmac.*, **1973**, **22**, 2591.
106. Kesner, L., Aronson, F. L., Silverman, M., and Chan, P. C., *Clin. Chem.*, **1975**, **21**, 353.
107. O'Gorman, L. P., *Clinica Chim. Acta*, **1969**, **23**, 247.
108. Taylor, J. A., *Clin. Pharmac. Ther.*, **1972**, **13**, 710.
109. Bush, I. E., *Adv. Clin. Chem.*, **1969**, **12**, 57.
110. Fecher, R., Chanley, J. D., and Rosenblatt, S., *Analyt. Biochem.*, **1964**, **9**, 54.
111. Broich, J. R., Hoffman, D. B., Goldner, S. J., Andryauskas, S., and Umberger, C. J., *J. Chromat.*, **1971**, **63**, 309.
112. Yeh, S. Y., Flanary, H., and Sloan, J., *Clin. Chem.*, **1973**, **19**, 687.
113. Reid, E., *in* Spencer, B., *Editor*, "Industrial Aspects of Biochemistry," North-Holland Publishing Co., Amsterdam, **1974**, p. 913.
114. Mattox, V. R., Litwiller, R. D., and Goodrich, J. E., *Biochem. J.*, **1972**, **126**, 533.
115. Schill, G., *in* Marinsky, J. A., and Marcus, Y., *Editors*, "Ion Exchange and Solvent Extraction," Volume 6, Marcel Dekker, New York, 1974, p. 1.
116. Morreal, C. E., Dao, T. L., and Lonergan, P. A., *Steroids*, **1972**, **20**, 383.
117. Passon, P. G., and Peuler, J. D., *Analyt. Biochem.*, **1973**, **51**, 618.
118. Cameron, J. D., *Clinica Chim. Acta*, **1974**, **56**, 307.
119. Michaelis, A. F., Cornish, D. W., and Vivilecchia, R., *J. Pharm. Sci.*, **1973**, **62**, 1399.
120. Leppard, J. P., and Reid, E., *A. Rep. Prog. Chem. B*, **1974**, **71**, 44.
121. Landgraf, W. C., *in* Carr, C. J., and Usdin, E., *Editors*, "The Phenothiazines and Structurally Related Drugs," Raven Press, New York, **1974**, p. 357.
122. Scott, C. D., Chilote, D. D., Katz, S., and Pitt, W. W., jun., *J. Chromat. Sci.*, **1973**, **11**, 96.
123. Blair, A. D., Forrey, A. W., Meijsen, B. T., and Cutler, R. E., *J. Pharm. Sci.*, **1975**, **64**, 1334.
124. Dell, D., *in* Reid, E., *Editor*, "Methodological Developments in Biochemistry. 5. Assay of Drugs and Other Trace Organics in Biological Fluids," ASP Biological and Medical Press B.V., Amsterdam, 1976, in the press.
125. Stevenson, R. L., and Burtis, C. A., *Clin. Chem.*, **1971**, **17**, 774.
126. Kissinger, P. T., Felice, L. J., Riggin, R. M., Pachia, L. A., and Wenke, D. C., *Clin. Chem.*, **1974**, **20**, 992.
127. Garland, W. A., Trager, W. F., and Nelson, S. D., *Biomed. Mass Spectrosc.*, **1974**, **1**, 124.
128. Schulten, H. R., and Games, D. E., *Biomed. Mass Spectrosc.*, **1974**, **1**, 120.
129. Anders, M. W., and Latorre, J. P., *J. Chromat.*, **1971**, **55**, 409.
130. Rosen, L., and Goodall, McC., *Am. J. Physiol.*, **1962**, **202**, 883.
131. Tyce, G. M., Sharpless, N. S., and Owen, C. A., jun., *Biochem. Pharmac.*, **1972**, **21**, 2409.
132. Persson, B.-A., and Karger, B. L., *J. Chromat. Sci.*, **1974**, **12**, 521.
133. Eksborg, S., Lagerström, P.-O., Modin, R., and Schill, G., *J. Chromat.*, **1974**, **83**, 99.
134. Khym, J. X., *Clin. Chem.*, **1975**, **21**, 1245.
135. James, H., *Analyst*, **1973**, **98**, 274.
136. Bauer, R., *Clin. Chem.*, **1974**, **20**, 917.
137. Booker, H. E., and Darcey, B., *Epilepsia*, **1973**, **14**, 77.
138. Joern, W. A., *Clin. Chem.*, **1975**, **21**, 1548.

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The Automatic Determination of Silicate Dissolved in Natural Fresh Water by Means of Procedures Involving the Use of Either α- or β-Molybdosilicic Acid

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This paper describes the practical application of Truesdale and Smith's (1975) fundamental re-appraisal of the conditions that lead to the formation of α- and β-molybdosilicic acid in aqueous solutions. Two procedures, one using the α-acid and the other the β-acid, are proposed. Both procedures are designed so as to enable silicate-silicon concentrations of between 0 and 1·0 mg l^{-1} to be determined, although slight adjustments to the manifold of the Technicon AutoAnalyzer II system would make both suitable for other concentration ranges. The appropriate "molybdenum blues" derived from the yellow acids are used.

Tests of the precision of both procedures were made at five concentration levels between 0 and 1·0 mg l^{-1} of silicate-silicon in distilled water. The maximum value of the coefficient of variation was 1·44 per cent. In other tests 27 samples of natural water from the upland area surrounding the sources of the rivers Wye and Severn were analysed by both methods. The results suggest that for these types of water the methods yield the same value for silicate concentration.

Strickland[1] found that two forms of molybdosilicic acid can be formed in mixtures of molybdate, acid and silicate. His discovery led to improvements in methods for silicate analysis; in particular, it demonstrated that conditions that allow mixtures of the acids to form should be avoided. Nevertheless, Strickland[1] did not supply an adequate description of the conditions under which the two acids are formed. Recently,[2] his belief that the critical factor that determines the form produced in any solution is the ratio of the concentrations of acid and molybdate used has been shown to be erroneous; it is now recommended[2] that the primary factors to be considered are the pH and molybdate concentration of the reaction mixture.

The use of an inappropriate fundamental framework with which to describe the behaviour of a system must eventually hinder, or even mislead, further investigation of the system. We believe that acceptance of Strickland's acid to molybdate ratio concept[1] has seriously retarded progress in several branches of molybdosilicic acid chemistry, including the analytical branch.[2] Perhaps the clearest example of this effect is that in which Hargis,[3] in the studies of the formation of the α-molybdosilicic acid, describes how he was compelled to adopt a contorted experimental design so as to satisfy the acid to molybdate ratio constraint. He stated that the ratio concept introduced a problem that is not encountered in otherwise analogous studies of the formation of molybdophosphoric and bismuthomolybdophosphoric acids.

We believe that the evolution of methods for silicate analysis[1,4–24] has been impeded for three reasons. Firstly, it has not been possible reliably to produce solutions that contain only one acid.[2] Secondly, it has been impossible to describe, and perhaps even investigate, the kinetics of molybdosilicic acid in a manner that would assist the analyst. Hargis[25] seems to be the only worker to have attempted such a study. However, his work was restricted to a narrow band of conditions that covered only β-molybdosilicic acid formation and consequently the results are of limited direct application to analytical problems. Thirdly, studies as yet unpublished[26] show that existing knowledge of the spectrophotometric characteristics of aqueous solutions of α- and β-acids is generally incorrect. In particular, in contrast to previous workers,[1,4,5,23] we find that the absorptivities of the yellow acids are not equal at any wavelength between 290 and 400 nm when examined under identical conditions. It is

not possible, therefore, to develop an analytical procedure in which use is made of the yellow acids and a wavelength between these limits, and which becomes independent of the composition of a mixture of the α- and β-acids in the manner described by Garrett and Walker.[4]

In this paper two automatic procedures for the analysis of dissolved silicate are presented; one procedure depends upon the formation of α-molybdosilicic acid, the other on that of β-molybdosilicic acid. An automatic α-acid method for application to fresh waters does not appear to have been proposed before. Both procedures are free from significant interference from phosphate-phosphorus at the concentrations likely to be encountered in most natural fresh waters, and both are designed to operate at room temperatures above 17·0 °C. The α-acid procedure is therefore freed from two major disadvantages that Morrison and Wilson[13] encountered with their manual α-acid procedure, namely, high temperatures and phosphate interference.

This study began because, in our experience, an existing automatic procedure[20] for silicate analysis (by the β-acid procedure) was prone to erratic behaviour, which could not be accounted for in terms of AutoAnalyzer manifold operation. Instead, it seemed that the poor reproducibility resulted from a lack of understanding of the chemistry involved, and therefore, that further development work was required. It now seems that the study will also assist in the eventual objective of obtaining a standard method for silicate analysis. Of course, a prerequisite of a truly standard method is the knowledge that it is accurate. The accuracy of silicate procedures must remain in doubt, however, until more is known about the reactivity to acid and molybdate reagents of both interfering substances and the possible silicate species present in natural waters. The latter aspect still requires clarification, especially as it has been shown that condensation of silicate monomers can occur in some natural waters, particularly during freezing.[27,28] At this stage, therefore, we suggest that there is a real need to test the concordance of two (or more) procedures on batches of samples, whenever possible. Such a comparison is presented in this paper.

Experimental

Apparatus

The approach described previously[2] was used to study the formation of the two molybdosilicic acids under various conditions. Absorbance was measured by means of a Hilger and Watts Uvichem 1600 spectrophotometer; for kinetic studies the output from this instrument was displayed on a Smith's Servoscribe recorder. An EIL, Model 23A, direct-reading pH meter, standardised at pH 1·0 and 4·0, was used. The pH 1·0 standard was prepared by mixing 25 ml of 0·20 M potassium chloride solution with 67 ml of 0·20 M hydrochloric acid; the pH 4·0 standard was prepared from buffer tablets (Burroughs Wellcome & Co.). No corrections have been applied for differences of ionic strength between standards and other solutions. AnalaR reagents were used throughout the study and distilled water was prepared by distilling tap water in a Manesty still. Automatic analysis was performed on a Technicon AutoAnalyzer II system that incorporated a digital read-out.

Results

Time Required for Formation of Molybdosilicic Acid

General consideration of the kinetics of molybdosilicic acid formation

For the reasons given above it should now be possible to make use of studies of the kinetics of molybdosilicic acid formation in order to improve methods of silicate analysis. Whereas the appropriate time of formation has previously had to be determined empirically,[2,14,24] it might soon be possible to apply a rate equation that describes the reaction time precisely in terms of the relevant variables: pH, molybdate concentration, temperature, etc. In an attempt to achieve this end we have examined the kinetics of the formation process. Although the detailed results of these studies will be published elsewhere, those of direct analytical application are presented here.

Preliminary investigations showed that the formation curves for both acids are given by

$$A_t = A_\infty (1 - e^{-kt}) \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where A_t and A_∞ are the absorbances of the solution at time t and ∞, respectively, and k is an apparent rate constant ($k > 0$). Thus, A_t approaches an asymptotic value A_∞

exponentially, as t tends to infinity. We have tested the appropriateness of this equation more than 100 times by forming the molybdosilicic acids in mixtures containing silicate-silicon (0–3·00 mg l^{-1}) and molybdate-molybdenum (0–0·050 M) at appropriate pH values[2] between 0·8 and 4·8. In each instance the graph depicting $\log_{10}(A_\infty - A_t)$ *versus* time was linear with a correlation coefficient greater than 0·98. From equation (1) it follows that the precise time (t_f) required for a given fraction (f) of reaction is

$$t_f = \frac{-2{\cdot}303}{k} \cdot \log_{10}\left(1 - \frac{A_t}{A_\infty}\right) = \frac{-2{\cdot}303}{k} \cdot \log_{10}(1 - f) \quad .. \quad .. \quad (2)$$

Support for the validity of equation (1) can be drawn from two sources that were not available to us at the time we performed our work. Firstly, from studies of the initial formation rates of molybdosilicic acids, Hargis[25] concluded that the formation reactions are first order with respect to silicate concentration, an observation compatible with equation (1). Secondly, Garrett and Walker[4] found in their investigations that graphs of $\log(A_\infty - A_t)$ *versus* time were linear. In both sets of work it appears that the β-acid was formed, although some doubt must remain as Strickland's acid to molybdate ratio was used to describe the conditions. Also, these earlier reports, as well as being limited to β-acid formation, covered a much narrower range of pH and molybdate concentrations than has been tested in this work.

Specific considerations

In order to obtain rapid formation of the molybdosilicic acids during analysis, the pH of the reaction mixtures used in the β- and α-acid procedures should be kept as close as possible to 1·8 and 4·0, respectively. There are two reasons for this requirement. Firstly, the pH limits for the α- and β-acid production are 4·0 and 5·0 and 0·8 and 1·8, respectively.[2] Secondly, as our unpublished studies of the formation kinetics have shown, between the aforementioned limits the formation rate of the β-acid increases with increasing pH and the formation rate of the α-acid decreases with increasing pH.

In order to accommodate the required buffering capacity as well as a rapid formation of molybdosilicic acid, we have chosen to allow the pH of the reaction mixture to vary between 1·4 and 1·8 in the β-acid procedure, and between 4·0 and 4·2 in the α-acid procedure. Accordingly, the maximum time required for formation of molybdosilicic acid has been found by using equation (2) and the apparent rate constants obtained at pH values of 1·4 and 4·2 for β- and α-acid procedures, respectively.

A molybdate concentration of 0·050 M in a reaction mixture has been adopted in the recommended procedures so as to take advantage of a high rate of molybdosilicic acid formation. Our unpublished studies show that the rate constant for β-molybdosilicic acid formation rises rapidly over the range of molybdate concentrations between 0 and 0·025 M. Thereafter, the rise in rate constant for each increment of molybdate becomes progressively smaller; at 0·050 M it is less than 1/16th of the rise obtaining at low molybdate concentrations. The rate of formation of the α-acid behaves in a similar manner when the molybdate concentration is changed in this way. We have not used still higher concentrations of molybdate in the reaction mixture because Strickland[1] has shown that they can produce difficulties during the reduction of the molybdosilicic acid with tin(II) chloride.

For our purposes a procedure that could accommodate a concentration range of 0–1 mg l^{-1} in the sample was required. In this range the maximum resolution that can be expected from the AutoAnalyzer is approximately 2 in 1000 μg l^{-1}. (This constraint is imposed by both the thickness of the line the recorder draws and the size of the chart used.) Accordingly, a reaction time that yields 99·8 per cent. of the total yield of molybdosilicic acid ($f = 0{\cdot}998$) was adopted. Measurements of the kinetics of molybdosilicic acid formation at pH values 1·4 and 4·2, temperature 17·0 °C (lowest likely operating temperature) and molybdate concentration equal to 0·050 M showed that the minimum reaction periods for α- and β-acid procedures operating under these conditions are 10·6 and 4·7 min, respectively.

The effect of sodium chloride upon the rate of formation of the molybdosilicic acids has been studied. The presence of 100 mg l^{-1} of sodium chloride in the reaction mixtures in which the molybdosilicic acids are formed (molybdate-molybdenum concentration 0·050 M) had an insignificant effect on the rate constants (Table I). The small differences of up to 6 per cent. can be accounted for by slight variations in pH of the reaction mixtures. After

allowing for the amount that the sample contributes to the reaction mixture it is found that the α- and β-acid procedures will tolerate at least 363 and 535 mg l^{-1} of sodium chloride, respectively, in the sample.

TABLE I

EFFECT OF EITHER SODIUM CHLORIDE OR PHOSPHATE ON THE RATE OF FORMATION OF THE MOLYBDOSILICIC ACIDS

Reaction mixture composition: 0·050 M molybdate-molybdenum (pH, 3·9–4·0 for the α-acid; 1·5–1·6 for the β-acid); the α-acid mixture was 0·1 N in sodium acetate and 0·1 N in acetic acid.

Concentration of sodium chloride added/mg l^{-1}	Concentration of phosphate-phosphorus added/mg l^{-1}	Apparent rate constant/min^{-1}	
		α-Acid	β-Acid
0	0	0·94, 0·93	1·58, 1·57
100	0	0·91, 0·89	1·55, 1·50
0	4·0	0·89, 0·91	1·49, 1·49

Buffering

A fixed set of reagents and mixing regimes is used in each automatic method. It is essential, therefore, to ensure that likely variations in the acidity (alkalinity) of the samples does not give rise to intolerable pH levels in the reaction mixture. Buffering is desirable and probably essential in the α-acid procedure in which the concentration of acid in the reaction mixture is low (pH approximately 4·0). The β-acid procedure is inherently less sensitive to changes in acidity or alkalinity of the samples because the acid concentration of the reaction mixture is high (pH approximately 1·5). Therefore, with most natural waters a pH buffer is not necessary in the β-acid procedure. However, if for any reason a greater range of acidity (alkalinity) is anticipated additional buffering might be necessary; dichloroacetic acid ($pK_a = 1·5$) seems likely to be suitable.

In the present work, the reaction mixture of the α-acid procedure is buffered at pH 4·0. There are two reasons for choosing this pH value, which is at the lower end of the range appropriate for production of the α-acid at room temperature. Firstly, it is possible to take advantage of the higher rates of formation of the α-compound that occur at this pH. The reaction period of 10·6 min can easily be accommodated on an AutoAnalyzer and its use circumvents the need for the heating that has been recommended in some manual α-acid methods.[5,6,13] Secondly, both alkaline and acidic natural waters can be accommodated in a single procedure if the pH of the buffer is biased towards increased acidity in this way. The buffering capacity of 1 l of the reaction mixture with the recommended composition is such that addition of $1·4 \times 10^{-2}$ equiv of acid or alkali changes the pH by 0·2 unit. In the recommended automatic procedure, this pH change will therefore be produced by a sample containing $5·1 \times 10^{-2}$ equiv l^{-1} of acid or alkali. This buffering capacity appears to be sufficiently large to cope with the variable acidity (alkalinity) of most natural waters.

Titrations of 0·050 M molybdate solution with 0·5 N hydrochloric acid showed that $4·8 \times 10^{-2}$ equiv of acid are required to change the pH of 1 l of the reaction mixture for the β-acid procedure from 1·4 to 1·8. The β-acid procedure recommended here can therefore tolerate natural waters with alkalinities (to pH 1·4) of approximately 0·24 equiv l^{-1}. This buffering capacity seems to be sufficiently large for most natural fresh waters.

Reduction

Several different reducing agents have been used in conjunction with molybdosilicic acids. We found that tin(II) chloride is an excellent reagent for automatic analysis provided that its exposure to air is limited, and for this purpose we prefer it to the metol(*p*-methylaminophenol sulphate) - sulphite reducing agent used by Brewer and Riley[15] because the latter requires high temperatures and a lengthy reaction period. It is also preferable to the 1-amino-2-naphthol-4-sulphonic acid reagent because, as Morrison and Wilson[11] have shown, the absorptivity of the α-acid product reduced with tin(II) chloride is 20 per cent. higher.

A tin(II) chloride concentration of $1·5 \times 10^{-3}$ N in the final reaction mixture is satisfactory, and the yield of reduction products obtained by the use of $1·0 \times 10^{-3}$ and $2·0 \times 10^{-3}$ N concentrations is probably similar (Table II). Although with these concentrations the absorbances (at 660 nm for α- and β-acids, 740 nm for the α-acid and 790 nm for the β-acid) of the blank solutions (0·0 mg l^{-1} of silicate-silicon) were identical, the reaction mixtures

were of different colours. Whereas with $2{\cdot}0 \times 10^{-3}$ N tin(II) chloride the blank for β-molybdosilicic acid was an intense golden colour, that with $1{\cdot}0 \times 10^{-3}$ N tin(II) chloride was only faintly brown. Similarly, the blanks for α-molybdosilicic acid were of noticeably different shades of green.

TABLE II

EFFECT UPON THE ABSORBANCE OF THE FINAL MIXTURE OF CHANGING THE TIN(II) CHLORIDE CONCENTRATION

Distilled water was used in the reference beam of the spectrophotometer.

	Reduced β-molybdosilicic acid		Reduced α-molybdosilicic acid	
Tin(II) chloride concentration*/N	$1{\cdot}0 \times 10^{-3}$	$2{\cdot}0 \times 10^{-3}$	$1{\cdot}0 \times 10^{-3}$	$2{\cdot}0 \times 10^{-3}$
Absorbance at 660 *nm* (1-*cm cell*)				
Increment†	0·355	0·355	0·521	0·519
Blank	0·015	0·023	0·011	0·019
Absorbance at 790 *nm* (1-*cm cell*)				
Increment†	0·712	0·701	—	—
Blank	0·028	0·031	—	—
Absorbance at 740 *nm* (1-*cm cell*)				
Increment†	—	—	0·624	0·620
Blank	—	—	0·010	0·018

* In each instance, a 1·00- or 2·00-ml aliquot of 0·050 N tin(II) chloride solution was added to a mixture of 25·0 ml of a molybdosilicic acid solution (2·00 or 0 mg l^{-1} of silicate-silicon) and 25·0 ml of 2·0 N sulphuric acid, which had stood for 2 min. When necessary extra distilled water was added to compensate for differences in volume.

† The increment in absorbance for 2·00 mg l^{-1} of silicate-silicon.

With the recommended conditions the reduction of β-molybdosilicic acid is almost complete within a few seconds at 17 °C. Thereafter, a slight decrease in absorbance (at 660 or 790 nm) of approximately 1 per cent. min^{-1} is observed for at least 5 min. In contrast, the reduction of the α-molybdosilicic acid is a slower process. Nevertheless, after 2 min the rate of increase in absorbance (at 660 or 740 nm) was less than 1 per cent. min^{-1}. Therefore, the periods allowed for reduction, 1·5 and 2·8 min in the β- and α-acid procedures, respectively, are long enough. Further, slight variations in these times produced by the AutoAnalyzer will not affect the reproducibility significantly.

The stability of the tin(II) chloride reagents has been investigated, and the stock 1·0 N solution was found not to deteriorate during storage for 6 months in a stoppered bottle. Although 0·01 N tin(II) chloride solution, prepared by diluting a stock of 1·0 N solution [in concentrated hydrochloric acid (11·2 N)] with distilled water, did not deteriorate during storage for 50 h in stoppered bottles, it deteriorated markedly when stored in an open beaker for 24 h. The tests were conducted using both α- and β-molybdosilicic acids. During the experiments sub-samples of a reservoir (1 l) of yellow α-molybdosilicic acid solution (approximately 2 mg l^{-1} of silicon) that were treated with 2 N hydrochloric acid and reduced with tin(II) chloride from a stoppered bottle returned a mean absorbance (740 nm, 1-cm cell) of 0·618 with a standard deviation of 0·002. On the 50th hour, tests were also performed on a β-molybdosilicic acid solution using both the 50 h old batch of 0·01 N tin(II) chloride solution as well as a batch prepared from a fresh 1·0 N stock solution. The absorbances (810 nm, 1-cm cell) of the reduced products were identical.

The above results show that the 0·01 N tin(II) chloride reagent must not be stored in an open beaker. Therefore, in order to avoid deterioration of the reagent during routine work we store it in a partially sealed 100-ml standard flask. The solution is drawn through a glass tube that passes through a rubber bung to the bottom of the flask; compensating air enters the flask through a second fine-bore tube. This storage regimen has proved to be reliable during extensive usage of both α- and β-acid procedures; the response obtained from standard silicate solutions has not decreased systematically during any one day's analysis.

Interference by Phosphate

Phosphate-phosphorus can combine with molybdate to produce molybdophosphoric acid. This reaction takes place spontaneously under the conditions used in the procedures recom-

mended here and can interfere (Table III). With the β-acid procedures this interference has previously been overcome by destroying the molybdophosphoric acid by addition of oxalic or tartaric acid. However, Morrison and Wilson[13] state that they were unable to overcome the interference in their manual α-acid method by this means. In contrast, Grasshoff[21] succeeded in overcoming the interference in his α-acid procedure for sea water by adding oxalic acid. Both of the procedures presented here have been freed from most of the interference from up to 7·0 mg l^{-1} of phosphate-phosphorus in the samples.

Table III

Suppression of phosphate interference by means of oxalic acid: the over-all effect

In each instance 3·0 ml of 50·0 g l^{-1} oxalic acid solution (or 3·0 ml of distilled water) were added to a mixture of 25·0 ml of molybdosilicic acid (2·00 or 0·00 mg l^{-1} of silicon) and 25·0 ml of 2·0 N sulphuric acid. After 2 min 1·00 ml of 0·050 N tin(II) chloride solution was added to give the molybdenum blue. Aliquots (25·0 ml) of molybdophosphoric acid (2·00 or 0 mg l^{-1} of phosphorus) were treated similarly.

	Sample identity	Absorbance (1-cm cell)			
		α-Acid procedure		β-Acid procedure	
		660 nm	740 nm	660 nm	790 nm
	Without oxalic acid				
	Blank	0·008	0·007	0·009	0·009
Increments	Silicate solution (2·00 mg l^{-1} of Si)	0·502	0·602	0·323	0·688
Increments	Phosphate solution (2·00 mg l^{-1} of P)	0·558	0·588	0·547	0·483
Increments	Silicate + phosphate	1·110	1·218	0·858	1·155
	With oxalic acid				
	Blank	0·016	0·012	0·002	0·001
Increments	Silicate solution (2·00 mg l^{-1} of Si)	0·475	0·588	0·321	0·690
Increments	Phosphate solution (2·00 mg l^{-1} of P)	0·014	0·012	0·004	0·001
Increments	Silicate + phosphate	0·471	0·589	0·320	0·681

In our exploratory experiments on the suppression of phosphate interference, approximately 1·0 mg l^{-1} of phosphate-phosphorus was maintained in the complete reaction mixture used for producing the blue, reduced molybdosilicic acids. The molybdosilicic and molybdophosphoric acids were allowed 10 min to develop at either pH 4·0 or 1·7 and room temperature (approximately 20 °C). Whereas the addition of 3·0 g l^{-1} of oxalic acid (in the final mixture) changed the blanks only slightly, it affected the increment in absorbance yielded by a given amount of silicate-silicon. The increment in absorbance obtained in the α-acid procedure decreased by approximately 6 per cent. (Table III). The reason for this effect is not known although several experiments, including some in which the absorbance (at 390 nm) of the yellow α-molybdosilicic acid was studied, were performed. Nevertheless, the experiments showed that the effect, such as it is, occurs rapidly after the oxalic acid is added and that afterwards the increment does not change significantly (Table IV). The behaviour in the β-acid procedure is different. The increment in absorbance yielded by a given amount of silicate varies with the period allowed to elapse between the addition of oxalic acid and tin(II) chloride. However, it appears (Table IV) that the addition of oxalic acid does not affect the magnitude of the initial value of the increment. As the absorbance, at both 660 and 790 nm, of the blue derivative decreases with increased time of exposure to oxalic acid (Table IV), it seems that some of the β-molybdosilicic acid decomposed; transformation of β-acid into α-acid would have led to an increase in the absorbance at 660 nm. Further investigation of these phenomena was suspended as they appear to be under control in the automatic procedures recommended here; the procedures yield reproducible results as well as linear calibration graphs. Notwithstanding the existence of the above effects, the results in Tables III and IV suggested that oxalic acid can suppress the tested phosphate interference. Other tests showed that with only 2·0 g l^{-1} of oxalic acid in the final mixture, some interference remained after 2 min in both methods. It seemed advisable, therefore, to use the higher concentration of 3·0 g l^{-1}.

Although the above experiments suggested that phosphate interference had been suppressed satisfactorily, tests with the automatic α-acid and β-acid procedures demonstrated a residual

TABLE IV

SUPPRESSION OF PHOSPHATE INTERFERENCE BY MEANS OF OXALIC ACID: EFFECT OF VARYING THE TIME OF EXPOSURE OF THE MOLYBDOSILICIC ACIDS TO OXALIC ACID

In each instance 10·0 ml of 100 mg l^{-1} silicate-silicon solution (or 10 ml of distilled water in the case of a blank) were added to 500 ml of acidified molybdate (0·050 M molybdenum) solution at either pH 1·6 (for β-acid) or pH 4·0 (for α-acid). Each 510-ml aliquot was allowed to stand for 10 min before 500 ml of 2 N sulphuric acid were added and the solution divided into two 500-ml portions. One portion was treated with 30·0 ml of oxalic acid (50 g l^{-1}) and the other with 30·0 ml of distilled water. After various periods of time, 26·5-ml aliquots of each mixture were treated with 0·5 ml of 0·05 N tin(II) chloride solution and the absorbance (1-cm cell) of each solution was measured. The mixtures that were not treated with oxalic acid are controls; with the β-acid they show the rate of transformation to the α-acid.

α-Acid			β-Acid		
	Absorbance			Absorbance	
Exposure time/min	660 nm	740 nm	Exposure time/min	660 nm	790 nm
With oxalic acid—					
4	0·440	0·539	5	0·327	0·673
10	0·442	0·539	12	0·322	0·653
15	0·443	0·542	15	0·320	0·644
20	0·442	0·539	20	0·318	0·634
25	0·442	0·540	25	0·315	0·621
30	0·444	0·539	30	0·315	0·613
35	0·444	0·540	35	0·311	0·600
40	0·446	0·540	40	0·310	0·591
45	0·448	0·542	45	0·310	0·582
50	0·451	0·539	50	0·309	0·582
			55	0·307	0·570
			60	0·305	0·559
			83	0·298	0·522
Without oxalic acid—					
2	0·467	0·552	10	0·333	0·667
23	0·462	0·548	18	0·330	0·663
42	0·468	0·551	42	0·333	0·641
			62	0·332	0·628

interference when the above amounts of oxalic acid were used. The interference was greater in the α-acid procedure, where samples of distilled water containing 0, 1, 2, 4 and 7 mg l^{-1} of phosphate-phosphorus produced a response equivalent to 0, 120, 170, 190 and 200 μg l^{-1} of silicate-silicon, respectively. The non-linear relationship between response and concentration excludes the possibility of silicate contamination of the phosphate reagents. Moreover, that argument is also untenable as phosphate solutions prepared from potassium dihydrogen orthophosphate, dipotassium hydrogen phosphate and sodium hexametaphosphate gave similar responses. Impurities of another type, however, cannot be dismissed. It is possible, therefore, that the behaviour observed is limited to certain phosphate reagents and that the phosphate in natural waters would not create the problem during analysis. Nevertheless, as the possibility of an interference by naturally occurring phosphate could not be excluded, it became essential to suppress the residual interference as far as possible. This suppression was accomplished by adding phosphate to the molybdate reagent and increasing the oxalic acid concentration above that used in the exploratory tests. The increase is approximately 6-fold for the α-acid procedure and 2·5-fold for the β-acid procedure. With this arrangement, up to 7 mg l^{-1} of phosphate-phosphorus in a sample produces a response in both procedures equivalent to less than 3 μg l^{-1} of silicate-silicon. We also found that our earlier routine use of a detergent (Ultra-wet 60-L) with the α-acid procedure was neither necessary nor desirable with the later arrangement of reagents. The later arrangement of reagents seemed to possess its own detergent action and very smooth recorder traces were obtained. The inclusion of Ultra-wet 60-L detergent in the molybdate reagent led to intolerable disruption of the recorder traces. The nature of the residual compound responsible for the phosphate interference is still unknown to us.

Kinetic studies showed that under the conditions used in the procedures the rate of formation of α- and β-molybdosilicic acids is not changed significantly by the presence of

the amount of phosphate-phosphorus used in the above experiments. In each instance (Table I) the rate constants agreed to within 6 per cent., the difference being probably due to slight variations in the pH of the reaction mixtures. Therefore, competition between phosphate and silicate for molybdate, as apparently observed by Wilson,[14] does not occur in these procedures. Presumably this is so because, here, a relatively high concentration[10] of molybdate-molybdenum has been used in the reaction mixture.

Only fresh solutions of phosphate, prepared from disodium hydrogen orthophosphate, were used in the above tests. It was found that when stock phosphate solutions are stored in glass bottles they become contaminated with silicate. In studies of these interferences such contamination can lead to erroneous conclusions if its presence is not suspected.

Sample Blank

When samples contain different amounts of background material it is essential to make a correction. Ideally, for a given sample this correction would be obtained by analysing a second aliquot from which all silicate had been removed but was otherwise of exactly the same composition as the parent material; in this way the correction would compensate for chemical interferences as well as background coloration. Unfortunately, such treatments for the removal of silicate do not exist and alternative approaches, which compensate only for background coloration, have to be used. Here, it is recommended that sample blanks be obtained by repeating the analysis under exactly the same conditions, except that distilled water is used in place of the molybdate reagent. By this means, the formation of molybdosilicic acid is prevented, and the conditions under which background coloration is measured closely resemble those under which the reduced molybdosilicic acid concentration is measured.

Filtration

In order to avoid introducing a significant turbidity blank into the analysis samples should be filtered. In this work samples were filtered through Millipore membrane filters, of 0·8 μm average pore diameter, which were mounted on a Millipore Swinnex filter holder, the top of which had been modified to accept a polypropylene tube that was 3·5 cm in diameter and 12 cm long, into which the sample was poured. Provided that the membranes were washed before filtration commenced, standard silicate solutions (0 and 1·00 mg l^{-1} of silicon) filtered through this apparatus did not suffer significant deterioration; the apparatus seems to be free from the kind of contamination problem that Liss and Spencer[22] encountered with some other types of filtration apparatus. However, when the membrane filters were not washed, 15-ml aliquots of the standard solutions became contaminated with approximately 10 μg l^{-1} of silicate-silicon.

Distilled Water

If the distilled water used for calibration purposes contains a significant amount of dissolved silicate-silicon the results for the analyses will be systematically too low. In order to prevent such errors the distilled water should be repeatedly re-distilled in an all-metal still until the results for the analyses carried out with water from consecutive distillations are in agreement. We have applied this approach here and have found that a single distillation of the relatively hard Wallingford water was sufficient.

Precision

The precision of each procedure was tested at five concentration levels between 0 and 1·00 mg l^{-1} by analysing five sets of 11 replicate samples of silicate-silicon in distilled water. The sets were taken in order of increasing concentration and were not randomised; each estimate of precision was therefore obtained under conditions which probably slightly favour a low standard deviation. The tests for the procedures were made on consecutive days, while the tests for any one procedure were completed within 3 h.

The results obtained (Table V) show that the precision of the two procedures is similar and within the levels usually expected of automatic methods that involve the use of the Technicon AutoAnalyzer II system. The maximum value of the coefficient of variation, and therefore the worst precision, was obtained during an analysis of the 200 μg l^{-1} sample by the α-acid procedure.

It is unlikely that the procedures would be operated with maximum range expansion as greater sensitivity could be more reliably obtained by a slight increase in the sample flow in the manifold. Nevertheless, the tests conducted here, with maximum range expansion (Table V), show that both procedures will tolerate range expansion without a serious loss of precision.

Calibration Graph

The calibration graphs for the range 0–1·0 mg l^{-1} are linear (Table V). With the 660-nm interference filters used in this work, the absorbance range extends between 0 and approximately 0·25. The use of 740-nm and 810-nm interference filters for the α- and β-acid procedures, respectively, would allow a greater absorbance range to be spanned.[2] In general, the use of these higher wavelengths has the further advantage that "sample blank" values are reduced.

TABLE V

PRECISION OF α- AND β-ACID PROCEDURES

(*a*) At recommended scale expansion (100 chart divisions ≡ 1000 μg l^{-1}); absorbance approximately 0 to 0·25.

Sample concentration/μg l^{-1} of silicate-silicon	α-Acid procedure		β-Acid procedure	
	Mean response (chart divisions)	Coefficient of variation, per cent.	Mean response (chart divisions)	Coefficient of variation, per cent.
200	19·4	1·44	19·5	0·72
400	39·2	0·66	39·1	0·46
600	59·4	0·45	59·2	0·27
800	79·3	0·33	79·4	0·15
1000	97·3	0·51	99·0	0·43

Linear regression of the calibration graphs gave $Y_\alpha = 0{\cdot}098x + 0{\cdot}15$

$Y_\beta = 0{\cdot}100x - 0{\cdot}55$

where Y_α and Y_β are the responses (chart divisions) for α- and β-acid procedures, respectively, and x is the silicate-silicon concentration (μg l^{-1}). In both instances analysis of variance showed no evidence for non-linear components in the linear regression.

(*b*) With maximum scale expansion, *i.e.*, absorbance approximately 0 to 0·05.

	α-Acid procedure	β-Acid procedure
Full scale equivalent to	215 μg l^{-1}	254 μg l^{-1}
Mean response for 200 μg l^{-1} (chart divisions)	93·0	78·7
Coefficient of variation, per cent.	2·4	0·56

Base-line stability

With the α-acid procedure we have been unable to detect any systematic drift in the base-line. However, the base-line and peaks of the β-acid procedure were found to drift at approximately 3 chart units h^{-1} (in absorbance, 0·006 per hour). We believe that the drift is caused by changes in the acidified molybdate solution; tests showed that the other reagents were not responsible for the drift. It seems that it could be eliminated by using separate acid and molybdate reagents. However, we prefer to tolerate this low rate of drift and thereby take advantage of the precise pH control afforded by a single reagent.

Experience has shown that it is inadvisable to substitute hydrochloric acid for the recommended sulphuric acid. When we made this substitution, it was found that both the base-line and the peaks drifted excessively for 1 h or more. We are unable to offer a complete explanation for this phenomenon but believe that it is caused by an interaction between hydrochloric acid and the pump-tube material; earlier work[2] had shown that this change in mineral acids does not change the amount of molybdosilicic acid formed.,

Comparison of the Procedures

The α- and β-acid procedures were compared by analysing 27 filtered samples of water from the upland area that surrounds the sources of the rivers Severn and Wye; each sample was analysed in triplicate. The pH values of the samples ranged between 3·2 and 5·0 and some samples that were taken from peat bogs contained appreciable amounts of humic materials (about 50 mg l^{-1}). A typical analysis of the samples gave the following results:

sodium 3·0 mg l^{-1}, potassium 0·5 mg l^{-1}, calcium 0·35 mg l^{-1}, magnesium 0·44 mg l^{-1}, chloride 2·0 mg l^{-1} and conductivity 70 $\mu\Omega^{-1}$ cm^{-1}. In order to accommodate the samples, full-scale deflection of the AutoAnalyzer recorder was fixed at 2·0 mg l^{-1} of silicate-silicon. The results of the silicate analyses are given in Table VI. (As no attempt was made to "round-off"

Table VI

Results and analysis of variance of results obtained during comparison of the α- and β-acid procedures using upland river waters

Concentrations in $\mu g\ l^{-1}$.

(*a*) Individual results

Sample No.	Procedure: α-Acid	Procedure: β-Acid	Difference between means, per cent.	Sample No.	Procedure: α-Acid	Procedure: β-Acid	Difference between means, per cent.
1	78	70		15	634	632	
	76	72	4·0		632	628	0·32
	76	70			632	626	
2	1474	1438		16	922	922	
	1472	1450	0·94		930	922	0·22
	1476	1452			928	924	
3	1080	1066		17	1230	1224	
	1074	1064	0·65		1230	1224	0·30
	1076	1058			1230	1220	
4	1054	1030		18	1026	1022	
	1058	1038	0·76		1026	1022	0·20
	1042	1038			1024	1020	
5	616	620		19	550	550	
	622	626	0·21		544	548	0·30
	626	626			542	548	
6	1388	1386		20	994	1000	
	1374	1390	0·31		1002	1000	0·03
	1378	1390			1006	1000	
7	1524	1546		21	606	600	
	1540	1558	0·23		598	596	0·33
	1560	1562			596	592	
8	104	124		22	1356	1352	
	98	118	9·4		1352	1356	0·05
	96	118			1360	1356	
9	1188	1204		23	648	638	
	1200	1214	1·3		636	634	0·47
	1200	1216			634	628	
10	818	840		24	1384	1382	
	824	838	0·92		1392	1384	0·17
	826	836			1390	1386	
11	402	412		25	1530	1532	
	400	410	1·4		1548	1542	0·02
	392	406			1542	1544	
12	1442	1452		26	1158	1146	
	1438	1456	0·30		1152	1138	0·55
	1460	1458			1146	1134	
13	278	284		27	1282	1276	
	260	274	1·6		1284	1274	0·29
	264	270			1282	1276	
14	870	862					
	880	872	0·57				
	886	872					

(*b*) Analysis of variance

Variation	Degrees of freedom	Mean squares	*F*-ratio
Samples	26	1 136 768	6204
Procedures	1	25·284	0·14
Procedures × samples interaction	26	183·22	5·82
Residuals (between triplicate variation) ..	108	31·46	—
Total	161		

the figures until statistical analysis had been completed, the results given in the table must not be taken to imply extraordinarily good precision.)

A "mixed effects" statistical model of the form

$$Y_{ijh} = \mu + S_i + m_j + (Sm)_{ij} + \epsilon_{ijk}$$

was used to describe Y_{ijh}, the value of the kth determination on the ith sample by the jth procedure in terms of an over-all mean μ, sample and procedure effects S_i, m_j ($i = 1, 2 \ldots 27$; $j = 1, 2$), an interaction term $(Sm)_{ij}$, and a residual expressing the deviation appropriate to the kth determination. Samples and procedures were regarded as random and fixed factors, respectively, in the senses used by Scheffé.[29] The analysis of variance is shown in Table V where the appropriate test of the hypothesis that procedures yield identical determinations is given by the variance ratio (procedures mean square divided by samples × procedures mean square). This analysis shows no significance so that the data are consistent with the hypothesis of no difference between results returned by the procedures.

An F-test of the procedures × samples mean square against the mean square between triplicate analyses shows that there is significant ($P < 0{\cdot}001$) procedures × samples interaction, thus demonstrating that the difference between means of triplicate determinations, obtained for each procedure, varies from sample to sample. Inspection of the differences between triplicate means shows, however, that 25 of the 27 samples gave very similar means for silicate content by the two procedures (differing by less than 1·6 per cent.); the bulk of the significant interaction is caused by two "outlier" samples, the differences between the triplicate means of which amount to 4·0 and 9·4 per cent. As there was no objective reason for discounting them, apart from their low silicate concentration, they were included in the whole analysis although their contribution to the interaction is disproportionately large. Taken over all samples, the mean silicate determinations by the α- and β-acid procedures were 950 ± 3 μg l^{-1} and 949 ± 3 μg l^{-1}, respectively.

Analytical Methods

Reagents for α-Molybdosilicic Acid Procedure

Standard silicate solution (0·500 g l^{-1} *of silicon*). Fuse 1·0696 g of silica (SiO_2) with 5·0 g of sodium carbonate in a platinum crucible. Dissolve the cooled melt in distilled water and make the total volume up to 1 l. This stock solution should be stored in a polythene vessel and aliquots diluted with distilled water to give working solutions.

Buffered molybdate reagent. To 12·2 g of AnalaR ammonium molybdate [$(NH_4)_6\,Mo_7O_{24}.4H_2O$] add 138 ml of a solution containing 136·0 g l^{-1} of sodium acetate trihydrate and 58 ml l^{-1} of glacial acetic acid (this latter solution is 1·0 M in both sodium acetate and acetic acid). Add distilled water to make the volume up to 950 ml. By adding 5·0 N sulphuric acid (approximately 12 ml) adjust the pH of the mixture to 4·0 using a pH meter. Add 25·0 ml of 100 mg l^{-1} phosphate-phosphorus solution (0·439 g l^{-1} of potassium dihydrogen orthophosphate). Add distilled water to make the volume up to 1 l. Filter the solution through a Millipore membrane (average pore diameter 0·8 μm). As a precipitate forms in the solution after about 24 h, it should be prepared immediately before use.

Sulphuric acid - oxalic acid reagent. Dissolve 50·0 g of oxalic acid in approximately 900 ml of distilled water. Add 78·5 ml of concentrated sulphuric acid (sp. gr. = 1·84) and, after cooling, add distilled water to make the volume up to 1 l.

Tin(II) chloride reducing agent. Prepare 100 ml of 1·0 N stock solution by dissolving 11·0 g of tin(II) chloride dihydrate ($SnCl_2.2H_2O$) in concentrated hydrochloric acid. This solution is stable for at least 6 months when stored in a stoppered bottle. When required, prepare 100 ml of a 0·01 N working solution by diluting 1 ml of the stock solution with distilled water. This working solution is stable for at least 3 d when stored in a stoppered bottle. Nevertheless, we recommend that it should be prepared freshly each day and stored in the flask described above.

Reagents for β-Molybdosilicic Acid Procedure

Acidified molybdate reagent. Dissolve 10·8 g of ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$] in approximately 950 ml of distilled water. Using a pH meter, adjust the pH of the solution to 1·40 by adding 5 N sulphuric acid (approximately 20 ml). Add 20·0 ml of 100 mg l^{-1}

phosphate-phosphorus solution (0·439 g l^{-1} of potassium dihydrogen orthophosphate). Add distilled water to give 1 l of solution. Filter the solution through a Millipore membrane (average pore diameter 0·8 μm) before use. As a precipitate forms in this solution after a few days, it should be prepared freshly each day.

Oxalic acid - sulphuric acid reagent. Dissolve 35·0 g of oxalic acid in approximately 700 ml of distilled water. Add 140 ml of concentrated sulphuric acid (sp. gr. 1·84), cool and make the volume up to 1 l with distilled water.

Tin(II) chloride reducing agent and silicate standard. As described above for the α-molybdosilicic acid procedure.

Procedure

Assemble the AutoAnalyzer II manifold according to the design given in Fig. 1 and specifications shown in Table VII. Ensure that the temperature of reagents and samples is higher than 17·0 °C. While pumping the reagents and distilled water as sample (sampler

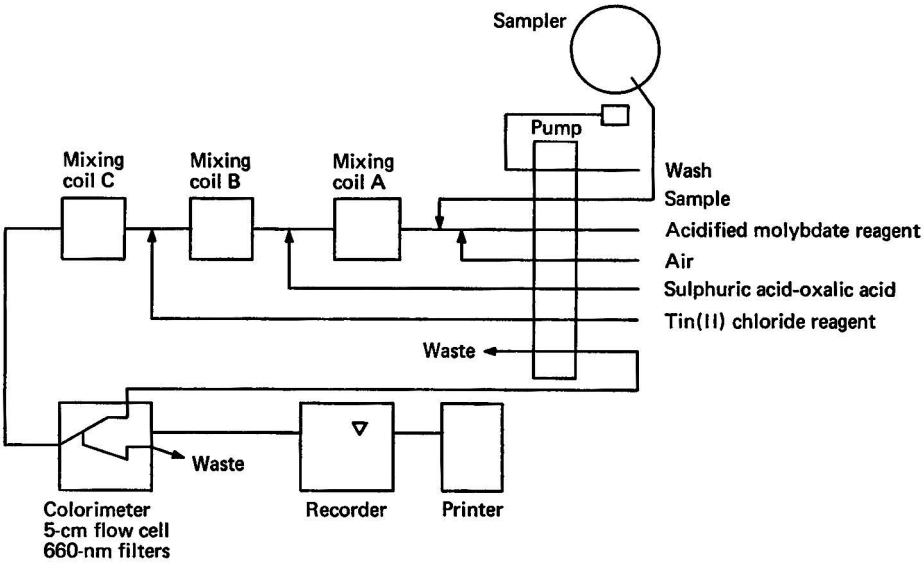

Fig. 1. Design of AutoAnalyzer II manifold (see Table VII for specifications).

TABLE VII

THE AUTOANALYZER II MANIFOLD DESIGN USED IN THE α- AND β-ACID PROCEDURES

	α-Acid procedure	β-Acid procedure
Pump tube (clear standard) diameter/in		
Wash	0·073	0·073
Sample	0·020	0·025
Acidified molybdate reagent	0·035	0·051
Air	0·025	0·025
Sulphuric acid - oxalic acid reagent	0·030	0·030
Tin(II) chloride reagent	0·020	0·025
Return line from colorimeter	0·045	0·056
Sample to wash ratio	8:1	5:1
Samples per hour	40	60
Time of mixing in coils/min		
A	10·6	4·7
B	1·3	1·8
C	2·8	1·5
Glass coils used in this study		
A	6 × 10 turns + 1 × 5 turns	5 × 10 turns
B	1 × 10 turns	2 × 10 turns
C	2 × 10 turns	1 × 10 turns

probe in wash position), set the absorbance at zero. When the base-line has stabilised (approximately 30 min are required in each instance), introduce samples and standards at the rate of 40 and 60 per hour for the α- and β-acid procedures, respectively. The sample to wash ratios, in the same order, are 8:1 and 5:1. A few minutes after the last sample has entered the AutoAnalyzer, substitute distilled water for the molybdate reagent and wait for a new setting of the base-line to be established; adjust the recorder to zero absorbance. Pass the samples through for a second time in order to obtain a peak that gives a measure of the sample blank. Read off the peak heights of the standards, samples (P_s) and sample blanks (P_b) after correcting for any slight drifting of the base-line in either of the two traces obtained. Calculate the gradient of the calibration graph (m) and then obtain each silicate-silicon concentration as follows

$$[\mathrm{Si}] = \frac{(P_s - P_b)}{m}\ \mu\mathrm{g\ l^{-1}}$$

where P_s and P_b are expressed in chart units and m in chart units $\mu\mathrm{g^{-1}\ l}$.

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References

1. Strickland, J. D. H., *J. Am. Chem. Soc.*, 1952, **74**, 862, 868 and 872.
2. Truesdale, V. W., and Smith, C. J., *Analyst*, 1975, **100**, 203.
3. Hargis, L. G., *Analyt. Chem.*, 1970, **42**, 1497.
4. Garrett, H. E., and Walker, A. J., *Analyst*, 1964, **89**, 642.
5. Ringbom, A., Ahlers, P. E., and Siitonen, S., *Analytica Chim. Acta*, 1959, **20**, 78.
6. Anderson, L. H., *Acta Chem. Scand.*, 1958, **12**, 495.
7. Luke, C. L., *Analyt. Chem.*, 1953, **25**, 148.
8. Hill, U. T., *Analyt. Chem.*, 1949, **21**, 589.
9. Baker, P. M., and Farrant, B. R., *Analyst*, 1968, **93**, 732.
10. Webber, H. M., and Wilson, A. L., *Analyst*, 1964, **89**, 632.
11. Morrison, I. R., and Wilson, A. L., *Analyst*, 1963, **88**, 88.
12. Morrison, I. R., and Wilson, A. L., *Analyst*, 1963, **88**, 100.
13. Morrison, I. R., and Wilson, A. L., *Analyst*, 1963, **88**, 446.
14. Wilson, A. L., *Analyst*, 1965, **90**, 270.
15. Brewer, P. G., and Riley, J. P., *Analytica Chim. Acta*, 1966, **35**, 514.
16. Govett, G. J. S., *Analytica Chim. Acta*, 1961, **25**, 69.
17. Abdullah, M. I., and Riley, J. P., "Automation in Analytical Chemistry," Technicon Symposium, Paris, 1966.
18. Chalmers, R. A., and Sinclair, A. G., *Analytica Chim. Acta*, 1965, **33**, 384.
19. Shapiro, L., *Res. U.S. Geol. Surv.*, 1974, **2**, 357.
20. Technicon AutoAnalyzer II, Industrial Method 66–71E, Technicon AutoAnalyzer Corporation, Basingstoke.
21. Grasshoff, K., *Deep Sea Res.*, 1964, **11**, 597.
22. Liss, P. S., and Spencer, C. P., *J. Mar. Biol. Ass. U.K.*, 1969, **49**, 589.
23. Langer, K., *Z. Analyt. Chem.*, 1969, **245**, 139.
24. Mullin, J. B., and Riley, J. P., *Analytica Chim. Acta*, 1955, **12**, 162.
25. Hargis, L. G., *Analyt. Chem.*, 1970, **42**, 1494.
26. Truesdale, V. W., and Smith, C. J., *Analyst*, 1975, **100**, 797.
27. Koyayashi, J., *in* Golterman, H. L., and Clymo, R. S., *Editors*, "Chemical Environment in the Aquatic Habitat," N.V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam, 1967, pp. 41–55.
28. Burton, J. D., Leatherland, T. M., and Liss, P. S., *Limnol. Oceanogr.*, 1970, **15**, 473.
29. Scheffé, H., "The Analysis of Variance," John Wiley & Sons, New York and London, Second Impression, March, 1961, p. 261.

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The Determination of Strontium-90 and Strontium-89 in Water without Separation of Strontium from Calcium

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A method has been developed for measuring strontium-90 and strontium-89 activities in water without separating the strontium from calcium. After evaporation of the sample to a convenient volume, the strontium plus calcium is purified radiochemically and an aqueous solution of their chlorides counted in a liquid scintillation spectrometer, which records the Čerenkov radiation produced. After a suitable period so as to allow the ingrowth of yttrium-90, the source is re-counted. From the two counts the activities of both strontium-90 and strontium-89 are calculated.

Activities as low as 0·1 pCi l^{-1} in natural waters as well as the higher levels in effluents from nuclear installations can be measured by this method.

Strontium-90 is among the most important radionuclides for which there is a need for extensive and precise surveillance, and easy and accurate methods for its determination are required. The assessment of strontium-89 levels of activity is of use in the tracing and dating of nuclear weapon tests and for measuring the age of fission products discharged from nuclear installations. The two radionuclides are pure beta-emitters and decay as follows:

$$^{90}\mathrm{Sr} \xrightarrow[T_{\frac{1}{2}} = 27{\cdot}7 \text{ years}]{\beta,\ 0{\cdot}54 \text{ MeV}} {}^{90}\mathrm{Y} \xrightarrow[T_{\frac{1}{2}} = 64{\cdot}0 \text{ h}]{\beta,\ 2{\cdot}27 \text{ MeV}} {}^{90}\mathrm{Zr} \qquad {}^{89}\mathrm{Sr} \xrightarrow[T_{\frac{1}{2}} = 52{\cdot}7 \text{ d}]{\beta,\ 1{\cdot}47 \text{ MeV}} {}^{89}\mathrm{Y}$$

They are most frequently counted by end-window Geiger counting and so the source has to be of low mass, and separation of the strontium carrier from calcium is essential. The most effective and commonly used method for achieving this separation involves the use of fuming nitric acid. This method can be used for calcium to strontium ratios as high as 200:1, giving good yields of strontium and at the same time effecting a high degree of decontamination from radionuclides. Its great disadvantage is the hazardous nature of the fuming nitric acid, litre volumes of which have to be used for samples high in calcium.[1] Strontium-90 has been determined by Čerenkov counting,[2,3] using the modern liquid scintillation counter for measuring the Čerenkov radiation.[4,5]

The application of this technique to the determination of strontium-89 and strontium-90 as described below has several advantages: the modern liquid scintillation counter is fully automatic and very reliable; the maximum beta-energy of calcium-45 is below the Čerenkov threshold in water (0·27 MeV), so there is no need to separate calcium from strontium; the source mass can be as high as 3·5 g in 20 ml; and the efficiency of counting is high. The main disadvantage is the high background count-rate. It should be noted that the difference in the Čerenkov spectra of the two radionuclides is not sufficient to enable either to be determined in the presence of the other at low levels of activity and, for this purpose, recourse is had to the ingrowth of yttrium-90 from strontium-90. Also, the determination of the two radionuclides without separating the strontium and calcium is possible only if the mixture of calcium and strontium can be adequately decontaminated from other radionuclides.

This step is achieved by a series of scavenges and the resulting precipitates that contain most of the gamma-emitters can, if determinations are required, be compressed into a disc, in the way that potassium bromide discs are prepared for infrared spectroscopy, so as to form a compact and precise source for counting on a lithium-drifted germanium detector. Ruthen-

ium must be scavenged very efficiently because of the high energy ($E_{max.} = 3{\cdot}5$ MeV) and the correspondingly high Čerenkov response of the ruthenium-106 - rhodium-106 pair of radionuclides.

Experimental

Apparatus

Packard "Tricarb" liquid scintillation spectrometer.
Perkin-Elmer 306 *atomic-absorption spectrophotometer.*
Centrifuges, centrifuge bottles of 250-ml capacity and centrifuge tubes of 40-ml capacity were used.

Reagents

All reagents should be of analytical-reagent grade.
Strontium carrier solution. A standardised solution of strontium chloride containing 50 mg ml^{-1} of strontium.
Yttrium carrier solution. A 20 mg ml^{-1} solution of yttrium oxide in diluted hydrochloric acid.
Ruthenium carrier solution. A solution of ruthenium(III) chloride in diluted hydrochloric acid containing 5 mg ml^{-1} of ruthenium.
Sodium chromate solution, 10 *g per* 100 *ml.*
Barium chloride solution, 4·5 *g per* 100 *ml.*
Sodium hydroxide solution, 50 *g per* 100 *ml.*
Hydrochloric acid (1 + 1).
Ammonium carbonate, solid.
Hydroxylammonium chloride, solid.
Ammonia solution, sp. gr. 0·88.

Procedure for Natural Waters

Take sufficient sample to meet the needs of accuracy and convenience; up to 100 l can be taken. Acidify with hydrochloric acid, add 5 ml of strontium carrier solution and 5 ml of barium chloride solution, then boil the mixture until the volume is reduced to 350 ml (if salts precipitate it is necessary to process a larger volume). Precipitate the hydroxides and carbonates by the addition of ammonia solution and solid ammonium carbonate, centrifuge the mixture and wash the precipitate twice with water. Dissolve the precipitate in hydrochloric acid and dilute the solution to 300 ml (silica may appear at this stage but will be removed later with the barium chromate).

Boil the solution so as to expel carbon dioxide and make it just alkaline to bromothymol blue with sodium hydroxide solution. (The presence of iron may mask the indicator, but at the desired pH the pale yellow colour of the iron will darken to brown.) Heat the solution to boiling, add 5 ml of sodium chromate solution and, after boiling for 1 min in order to coagulate the precipitate, filter it through a Whatman No. 30 filter-paper and wash the precipitate twice with water. Heat the combined filtrate and washings to boiling, add 5 ml of barium chloride solution, boil the solution, filter it and wash the precipitate twice with water.

To this combined filtrate and washings, add 2 ml each of ruthenium and yttrium carrier solutions and sufficient sodium hypochlorite solution (about 3 ml) to oxidise the ruthenium and produce a straw colour. Heat to just boiling, add sufficient solid hydroxylammonium chloride to reduce the ruthenium and chromate, then precipitate the ruthenium and the chromium with sodium hydroxide solution at pH 8 and boil the solution in order to coagulate the precipitate. Allow to settle, filter the mixture on a Whatman No. 541 filter-paper and wash the precipitate and filter-paper once with water. Make the solution just acid by the addition of hydrochloric acid, add 1 ml of ruthenium and 2 ml of yttrium carrier solutions and repeat the procedure from "and sufficient sodium hypochlorite . . . ."

Add 1 ml of yttrium carrier solution, followed by 1 ml of sodium hydroxide solution and sufficient solid sodium carbonate to precipitate the hydroxides and the carbonates. Centrifuge, wash the precipitate twice with water, dissolve it in the minimum amount of hydrochloric acid and dilute the solution to 70 ml. Boil the solution so as to expel the carbon

dioxide, add a few crystals of hydroxylammonium chloride and adjust the pH to 8 with sodium hydroxide solution. Boil the solution in order to coagulate the precipitate, filter it on a Whatman No. 541 filter-paper so as to remove the yttrium hydroxide and record the time at this stage, which is that at which yttrium-90 ingrowth starts. Wash the precipitate twice with water, acidify the filtrate with a few drops of hydrochloric acid and reduce the volume of the solution to about 22 ml by boiling it. Cool and pipette 20 ml into a plastic liquid scintillation vial.

Count and record the time at which counting is started. After a suitable ingrowth period of 1–10 d, depending on the activity present and the accuracy desired, re-count and again note the time at which the counting started.

Chemical yield

Determine the strontium and the calcium in the final counting solution, and if it is present in significant amounts, as is found with large volumes of samples of natural waters, also determine the strontium in the carrier-free solution. Make the determination by means of atomic-absorption spectrophotometry, using 1 per cent. lanthanum as a releasing agent. Calculate the strontium yield and from the calcium content of the counting solution assess the counting efficiencies of the three radionuclides from the counting efficiency graphs.

The average strontium yield is 60 per cent.

Counting efficiency graphs

The variation of the counting efficiency with the calcium content of the counting solution is small but may need to be taken into account.

Analytical-reagent grade calcium salts contain trace amounts of iron, which colour concentrated solutions and cannot be removed by direct precipitation as the hydroxide unless a carrier is present. Use yttrium as a carrier, then precipitate the calcium as carbonate, dissolve the precipitate in the minimum amount of hydrochloric acid and boil the solution in order to remove carbon dioxide. Prepare three sets of solutions containing 0, 1, 2 and 3·5 g of calcium per 20 ml of solution and dose one set with strontium-90, another with strontium-89 and the third with yttrium-90. Use high levels of activity for dosing so that counting times are short and ingrowth of yttrium-90 is slight. Count these solutions and then draw the counting efficiency graphs.

Typical ranges of efficiency, per cent., for 0–3·5 g of calcium are: strontium-90, 0·7–1·7; strontium-89, 35·8–38·4; and yttrium-90, 56·5–60·3.

Calculations

$$\text{Strontium-90 activity} = \frac{C_F - C_I D}{k \times R \times V \times 2{\cdot}22} \text{ pCi l}^{-1}$$

and

$$\text{Strontium-89 activity} = \left[C_I \left(\frac{1}{E_{89Sr}} + \frac{DK}{k} \right) - C_F \frac{K}{k} \right] \times \frac{1}{R \times V \times 2{\cdot}22} \text{ pCi l}^{-1}$$

(at the time of analysis) where

C_I = Initial counts per minute corrected for background.
C_F = Final counts per minute corrected for background.
G_I = Ingrowth factor for yttrium-90 at the mid-point of the first count.
G_F = Ingrowth factor for yttrium-90 at the mid-point of the final count.
D = Strontium-89 decay factor for the time elapsed between the mid-points of the initial and final counts.
E_{89Sr} = Fractional counter efficiency for strontium-89.
E_{90Sr} = Fractional counter efficiency for strontium-90.
E_{90Y} = Fractional counter efficiency for yttrium-90.
R = Fractional chemical yield of strontium.
V = Volume of sample in litres.
k = $E_{90Sr}(1 - D) + E_{90Y}(G_F - G_I D)$

$$K = \frac{E_{^{90}Sr} + G_I E_{^{90}Y}}{E_{^{89}Sr}}$$

Procedure for samples high in calcium

If the calcium content of the sample exceeds 3·5 g carry out a partial separation of the strontium from the calcium, modifying the method as follows. Dissolve the first carbonate precipitate in 1 + 1 acetic acid, dilute the solution to 300 ml with water and boil it in order to expel carbon dioxide. Adjust the pH of the solution to 6 by the addition of sodium hydroxide solution, precipitate calcium sulphate by addition of 10 ml of saturated sodium sulphate solution and heat the mixture to boiling. Cool, centrifuge it and, after washing the precipitate twice with water, add to the latter 20 ml of water and 3 g of sodium carbonate and heat the mixture on a boiling water bath for 30 min. Centrifuge it, wash the solids with water and then dissolve them in hydrochloric acid. Continue as under Procedure for Natural Waters, from "Dissolve the precipitate in hydrochloric acid . . . ."

This procedure has been applied successfully to samples containing 10 g of calcium.

Procedure for Effluents from Nuclear Installations

Usually a volume of between 10 and 400 ml is sufficient for these samples and it is not necessary to use the procedure for samples high in calcium.

Suitably scale down the procedure for natural waters and do not use less than 50 mg of strontium carrier in order to keep the activity of the strontium radionuclides to an acceptable level during the atomic-absorption spectrophotometric determination of the strontium. Also, for ease of handling and for effective scavenging, use an amount of ruthenium carrier of not less than 5 mg and of yttrium carrier of not less than 10 mg.

Results

Limits of Detection

The limits of detection of strontium-89 and strontium-90 are interdependent and the presence of either will raise the limit of detection of the other. In the absence of strontium-89 the limit of detection of strontium-90 is 0·8 pCi and in the absence of strontium-90 that of strontium-89 is 1·0 pCi. These limits have been calculated on the assumption that strontium-89 will be detected if the count-rate of the source is greater than three standard deviations above the background count-rate and that strontium-90 will be detected if the net ingrowth count-rate is greater than three standard deviations of the background count-rate. They relate to a counting time of 500 min, a chemical recovery of 60 per cent., a background count-rate of 13 counts min^{-1}, an ingrowth period of 8 d, a strontium-89 counting efficiency of 36 per cent. and a strontium-90 counting efficiency of 56 per cent.

Decontamination Factors

In order to determine the level of contamination of the final source by other radionuclides, decontamination experiments were carried out. Simulated water samples containing known

TABLE I

DECONTAMINATION FACTORS

Nuclide added	Decontamination factor as strontium-90	Decontamination factor as strontium-89
Uranium-238 to uranium-234 of uranium series	$>9{\cdot}1 \times 10^4$	$>8{\cdot}2 \times 10^4$
Radium-226 to lead-210 portion of uranium series	$9{\cdot}0 \times 10^3$	$1{\cdot}6 \times 10^5$
Lead-210 plus equilibrated daughters	$4{\cdot}6 \times 10^5$	$1{\cdot}9 \times 10^5$
Thorium-232 series	$>2{\cdot}4 \times 10^4$	$>2{\cdot}2 \times 10^4$
Ruthenium-106	$3{\cdot}8 \times 10^4$	$5{\cdot}0 \times 10^3$
Cerium-144	$>4{\cdot}9 \times 10^5$	$2{\cdot}1 \times 10^5$
Antimony-124	$>2{\cdot}0 \times 10^5$	$>1{\cdot}6 \times 10^5$
Antimony-125	$>1{\cdot}0 \times 10^5$	$7{\cdot}0 \times 10^4$
Cobalt-60	$2{\cdot}6 \times 10^6$	$3{\cdot}6 \times 10^5$
Caesium-137	$>1{\cdot}0 \times 10^5$	$3{\cdot}2 \times 10^4$
Barium-140	$1{\cdot}9 \times 10^5$	$5{\cdot}7 \times 10^3$

TABLE II

COMPARISON OF RESULTS FOR NATURAL WATERS

Results are expressed in pCi l^{-1}.

Calcium/ g per 100 l	Procedure	Period 1		Period 2		Period 3		Period 4	
		Strontium-90	Strontium-89	Strontium-90	Strontium-89	Strontium-90	Strontium-89	Strontium-90	Strontium-89
0·13	Described	2·19 ± 0·04	<0·29	2·41 ± 0·04	<0·13	1·87 ± 0·02	<0·15	2·43 ± 0·07	<0·22
	Previous	2·12 ± 0·03	<0·36	2·31 ± 0·05	<0·19	1·74 ± 0·08	<0·16	2·04 ± 0·04	<0·22
0·87	Described	1·14 ± 0·08	<0·53	1·12 ± 0·05	<0·20	1·00 ± 0·05	<0·30	1·14 ± 0·08	<0·31
	Previous	0·94 ± 0·03	<0·66	1·08 ± 0·04	<0·34	0·82 ± 0·03	<0·34	1·01 ± 0·04	<0·20
4·3	Described	0·07 ± 0·03	<0·21	0·14 ± 0·04	<0·15	0·07 ± 0·03	<0·17	0·16 ± 0·04	<0·16
	Previous	0·11 ± 0·01	<0·24	0·23 ± 0·01	<0·19	0·09 ± 0·01	<0·15	0·07 ± 0·01	<0·20
9·8	Described	0·38 ± 0·03	<0·22	0·39 ± 0·03	<0·14	0·35 ± 0·03	<0·27	0·36 ± 0·04	<0·15
	Previous	0·40 ± 0·02	<0·29	0·44 ± 0·03	<0·09	0·33 ± 0·02	<0·15	0·29 ± 0·01	<0·20
11·2	Described	0·19 ± 0·05	<0·38	0·19 ± 0·07	<0·34	0·08 ± 0·06	<0·36	0·19 ± 0·03	<0·15
	Previous	0·21 ± 0·01	<0·18	0·13 ± 0·01	<0·11	0·13 ± 0·01	<0·18	0·14 ± 0·01	<0·16

The confidence limits are for counting statistics only and are 1·64 × standard deviation for the plus or minus figures given under strontium-90 and 3 × standard deviation for the figures given under strontium-89.

activities of possible contaminants were taken through the procedure. The final source was counted and re-counted after 15 d, and decontamination factors were calculated from the ratio of the added activity of the parent nuclide only to the apparent activities of strontium-90 or strontium-89 in the sample. The results obtained are listed in Table I.

Natural Waters

Samples from five sites with a range of calcium concentrations were examined over four different periods by the described method and the method previously used in this laboratory.[7] The latter method consists in effecting separation by use of fuming nitric acid and Geiger counting an yttrium source for strontium-90 and a strontium source for strontium-89. The sample volumes were approximately 100 l, one quarter to one third of which was examined by the described method and the remainder by the previous method. The results obtained are listed in Table II.

Effluents from Nuclear Installations

Effluent samples were examined by the described method and by the method previously used for such samples in this laboratory, the latter consisting in carrying out separations with fuming nitric acid and, after removing the yttrium-90, Geiger counting a strontium source for comparison with a similarly prepared strontium-90 standard source, no allowance being made for the possible presence of strontium-89. The results obtained are listed in Table III.

TABLE III

COMPARISON OF RESULTS FOR NUCLEAR INSTALLATION EFFLUENTS

Results are expressed in nCi l^{-1}.

Previous method[7]: total radiostrontium activity as strontium-90	Described method	
	Strontium-90 activity	Strontium-89 activity
35·3	35·1	0·6
0·91	0·91	0·02
0·41	0·39	0·04
45·7	44·3	1·5
1·51	1·32	0·02
2·2	1·7	0·1

Neutron-irradiated Uranium

Natural uranium oxide was irradiated in a reactor in order to provide mixed fission products and was then dissolved in nitric acid. The activity of caesium-137 was measured so as to enable the strontium-90 and strontium-89 activities of the solution to be calculated from fission yield data. An aliquot of the solution was examined by the described method; activities were: strontium-90, calculated 0·61, described method 0·78 $\pm$ 0·24; strontium-89, calculated 62·4, described method 65·4 $\pm$ 0·1 nCi l^{-1}.

Time of Analysis

Processing times for natural waters, after evaporation, are very much reduced, a batch of two samples plus a blank taking 14 h as opposed to 14 h for each soft and 30 h for each hard water by the fuming nitric acid method. The maximum counting time that can conveniently be used in practice is 500 min, so two samples and a blank are counted in a 24-h period and, of course, it is best to count in cycles of 100 min per counting vial.

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References

1. "The Radiochemistry of Barium, Calcium and Strontium," National Academy of Sciences, National Research Council, Nuclear Science Series, NAS-NS 3010, Washington, D.C., 1960.
2. Randolph, R. B., *Int. J. Appl. Radiat. Isotopes*, 1975, **26**, 9.
3. Narrog, Von J., *Atompraxis*, 1965, **11**, 373.

4. Ross, H. H., *Analyt. Chem.*, 1969, **41**, 1260.
5. Parker, R. P., and Elrick, R. H., *in* Bransome, E. D., *Editor*, "The Current Status of Liquid Scintillation Counting," Grune and Stratton, New York, 1970.
6. "The Radiochemistry of Ruthenium," National Academy of Sciences, National Research Council, Nuclear Science Series, NAS-NS 3029, Washington, D.C., 1961.
7. Wood, R., and Richards, L. A., *Analyst*, 1965, **90**, 606.

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The Determination of Tellurium in Leaded Free-cutting Steels by Atomic-absorption Spectrometry

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A procedure is presented for the determination of tellurium within the range 0·005–0·08 per cent., with solvent conditions that permit its incorporation in a composite scheme that includes lead. In the preferred fuel-rich flame the background absorbance of iron and inter-element interferences are at a minimum.

Good recoveries of tellurium when added to solutions of leaded and stainless steels, together with satisfactory reproducibilities for samples and good agreements with results by the slower turbidimetric procedure, are shown.

Tellurium, up to 0·05 per cent., is added to leaded free-cutting steels in order to improve further their machinability. Consequently, its determination is often required.

Gravimetric and titrimetric methods for the determination of small amounts of tellurium are relatively insensitive and presuppose no interferences. Photometric determinations based on the formation of complexes with thiourea, iodide and sodium diethyldithiocarbamate are more precise,[1] while reduction with tin(II) chloride to colloidal tellurium in an acidic medium is the basis of a turbidimetric procedure that is commonly used within the steel industry.[2]

Previous workers[1] applied atomic-absorption spectrophotometry to the determination of tellurium within the range 0·0005–0·030 per cent. in steel and reported that the sensitivity was improved by extracting it into amyl acetate. Other workers,[3] using aqueous solutions, preferred its preliminary conversion into potassium hexaiodotellurate or tellurium diethyldithiocarbamate and extraction into isobutyl methyl ketone.

In more recent years the continued improvement in instrument and lamp design has enabled a more stable signal and superior sensitivity to be obtained for tellurium so that it can now be determined directly in acidic solution within the range 0–0·10 per cent. in steel. The object of the present work was therefore to develop a suitable procedure that would, at the same time, extend the composite scheme devised earlier for other elements.[4]

Experimental

The method developed for application to plain carbon, leaded free-cutting and stainless steels containing from 0·005 to 0·08 per cent. of tellurium is as follows.

Apparatus

A Techtron, Model AA4, atomic-absorption spectrophotometer, up-dated to the AA5 instrument by incorporation of the later type of read-out and burner units, was used with the following conditions.

Wavelength	214·3 nm
Lamp current	8 mA
Slit width	100 μm
Burner	Air - acetylene, 10-cm path length
Observation height	Light beam 1 mm above burner top
Fuel setting	Acetylene cylinder pressure, 10 lb in^{-2} and fuel flow adjusted to give minimum flame absorbance
Support setting	Air pressure, 30 lb in^{-2}; gauge reading, 18 lb in^{-2}
Damping	B
Scale expansion	× 2

Reagents

Hydrochloric acid, sp. gr. 1·16–1·18.

Nitric acid, sp. gr. 1·42.

Stock iron solution. Transfer 50 g of pure iron sponge into a 1·5-l beaker. Add 300 ml of hydrochloric acid (sp. gr. 1·16–1·18) and heat it gently so as to dissolve the iron. Cautiously oxidise it with nitric acid (sp. gr. 1·42) and boil off nitrous fumes. Replace any loss of acid with hydrochloric acid and add a further 200 ml. Cool the solution, dilute it to 1 l with water and filter.

Standard tellurium solution. Dissolve 0·1 g of Specpure tellurium metal in 20 ml of nitric acid (50 per cent. *V/V*) and dilute the solution to 1 l.

1 ml of solution ≡ 100 μg of tellurium.

Preparation of sample solution

Transfer 2 g of sample into a 150-ml conical beaker and add 40 ml of hydrochloric acid (50 per cent. *V/V*). Cover the beaker with a watch-glass and allow the mixture to digest until the sample has dissolved, then oxidise the iron with nitric acid (sp. gr. 1·42), adding one or two drops in excess. Boil the solution so as to expel nitrous fumes, cool it and dilute it to 100 ml in a calibrated flask. Filter if necessary.

Preparation of calibration solutions

Into each of a series of 50-ml calibrated flasks transfer 20 ml of stock iron solution. Add the tellurium fractions according to the following scheme, dilute each solution to the calibration mark and mix.

Standard tellurium solution/ml	Tellurium, p.p.m.	Tellurium, per cent.
0	0	0
1	2	0·01
2	4	0·02
4	8	0·04
6	12	0·06
8	16	0·08

Determination of Tellurium

Set the instrument according to the instrument conditions given above and spray the appropriate calibration solutions followed by the sample solution. Spray water between each test and set the zero while spraying water each time. Repeat this procedure. Prepare a calibration graph by plotting the absorbance readings against the equivalent percentages of tellurium. Convert the absorbance reading of the sample into tellurium percentage by reference to this graph.

Discussion

The choice of instrument parameters arose from the following considerations.

Flame Conditions

The absorbance by the flame at a wavelength of 214·3 nm was found to be dependent on the fuel to air ratio. The minimum absorbance was obtained by adjusting the acetylene flow while observing the meter needle, which resulted in a fuel-rich flame. Although the use of a weaker mixture was found not to affect the sensitivity or noise level, the background absorbance caused by iron(III) chloride was found to have its lowest value with the recommended flame conditions.

Sensitivity

The sensitivity in aqueous solution was found to be 0·18 p.p.m. and in the presence of iron (2 per cent. *m/V*), as in the method, to be 0·20 p.p.m. In order to cover the desired range of up to 0·080 per cent. of tellurium for a 2 per cent. *m/V* sample solution, a concentration of up to 16 p.p.m. was required. For tellurium contents of up to 0·040 per cent. a scale expansion

of × 2 was used. The noise level under these conditions was acceptable, requiring a low damping setting. The lamp current, wavelength and slit width recommended by the manufacturer were found to give the optimum results.

Matrix Effects

The apparent absorbance at 214·3 nm of a solution containing 2 per cent. *m/V* of iron, with the flame adjusted to give minimum absorbance, is equivalent to 1 p.p.m. of tellurium. This effect is attributed to light scattering by solid particles in the flame, which resulted from spraying solutions of higher salt concentrations. In the proposed method this effect is overcome by including 2 per cent. *m/V* of pure iron in the calibration solutions.

For leaded steels this apparent absorbance is identical with that found with pure iron for the same concentration. For stainless steels, however, the apparent absorbance is equivalent to 1·2 p.p.m. of tellurium. As the recovery of tellurium (see Interference Tests) is within the range of reproducibility of the method (*i.e.*, no change in sensitivity), this slight increase in apparent absorbance can be allowed for by processing a stainless-steel sample containing no tellurium and deducting any absorbance greater than the nil-point absorbance from that due to the sample solution, or by preparing calibration solutions containing element concentrations that are similar to those of the sample under test.

Interference Tests

As there is little published information on interferences in the direct determination of tellurium in solutions of steel samples, the effect of adding tellurium equivalent to 0·020 and 0·040 per cent. to solutions of leaded steels and also to stainless steels was studied. The analytical values for these samples are given in Table I. For the stainless-steel samples the increase in background absorbance was equivalent to 0·001 per cent. of tellurium in each instance, which was allowed for in the recovery tests.

TABLE I

ANALYTICAL VALUES FOR SAMPLES USED IN RECOVERY TESTS

Sample	Type	C	P	Mn	S	Si	Pb	Ni
1759	Leaded steel	0·08	0·087	1·12	0·27	<0·005	0·25	
8332		0·13	0·128	1·18	0·42		0·26	
BCS 235/2	18/9 Stainless steel + Ti	0·073	0·020	0·020	0·018	0·82		9·38
BCS 335	Stainless steel	0·093	0·018	0·94	0·023	0·67	0·0015	9·47
US 14		0·081	0·022	0·61	0·019	0·51	0·0005	7·95

Sample	Type	Cr	Cu	V	Co	Ti	Ta	Al
1759	Leaded steel							
8332								
BCS 235/2	18/9 Stainless steel + Ti	18·60	0·12	0·04	0·056	0·32		0·048
BCS 335	Stainless steel	18·45	0·11	0·04	0·034	0·46	0·0017	
US 14		18·22			0·046			

The results shown in Table II indicate that the method is free from inter-element interference.

TABLE II

TELLURIUM FOUND, PER CENT., AFTER ADDING (A) 0·020 AND (B) 0·040 PER CENT. TO SAMPLES OF LEADED AND STAINLESS STEELS

Sample	A	B
1759	0·020, 0·020	0·040, 0·040
8332	0·020, 0·0205	0·040, 0·0405
BCS 235/2	0·021, 0·020	0·0405, 0·0405
BCS 335	0·021, 0·020	0·0405, 0·040
US 14	0·021, 0·020	0·0405, 0·0405

Application and Reproducibility Tests

As no standardised samples were available for determining tellurium in steel, application and reproducibility tests were carried out on samples of "tellurised" leaded steels. The results obtained are given in Tables III and IV. All atomic-absorption spectrophotometric

TABLE III

RESULTS OF REPRODUCIBILITY TESTS FOR LEADED FREE-CUTTING STEEL SAMPLES

Sample	Tellurium found by chemical method, per cent.	Tellurium found by AAS, per cent.	Mean value	Reproducibility (95 per cent. $\pm$ 2 s)
3189 BB13	0·005	0·006, 0·0055, 0·005, 0·0055, 0·0045, 0·0045, 0·005, 0·004, 0·0055, 0·005	0·005	0·0012
3189 B13	0·010	0·012, 0·011, 0·012, 0·011, 0·009, 0·0115, 0·012, 0·0115, 0·0095, 0·0115	0·011	0·0021
1993 A5	0·035	0·033, 0·034, 0·0345, 0·034, 0·0345, 0·0345, 0·034, 0·0335, 0·0335, 0·033	0·034	0·0012
1993 B5	0·034	0·033, 0·034, 0·0355, 0·034, 0·034, 0·0345, 0·034, 0·035, 0·0335, 0·034	0·034	0·0014
1993 C5	0·029	0·028, 0·030, 0·030, 0·0295, 0·029, 0·029, 0·029, 0·030, 0·029, 0·029	0·029	0·0013

results given were obtained by following the complete procedure on separate occasions. The chemical values, provided by independent laboratories, were obtained by photometric measurement of the turbidity due to colloidal suspension of tellurium following reduction with tin(II) chloride.[2] The good agreement of the results with those obtained by the chemical procedure confirms that the method is accurate and gives adequate reproducibility.

TABLE IV

COMPARISON OF RESULTS OBTAINED BY CHEMICAL AND ATOMIC-ABSORPTION SPECTROPHOTOMETRIC METHODS

Samples of leaded free-cutting steel	Tellurium, per cent.	
	Chemical value	Value found by AAS
3140	0·018	0·017, 0·017
2206 A4	0·053	0·0515, 0·0515
2206 A10	0·041	0·0405, 0·042
2206 A14	0·036	0·037, 0·037
2206 A15	0·040	0·0405, 0·0415
4572 B1	0·047	0·046, 0·046
4572 B3	0·039	0·0395, 0·0405
4572 B9	0·048	0·046, 0·046
3958 M2	0·042	0·042, 0·042
3992 M2	0·036	0·0365, 0·0355
3992 M3	0·036	0·036, 0·036
3355 4	0·040	0·040, 0·040
BOT 1	0·024	0·024, 0·023
BOT 2	0·027	0·026, 0·025

Conclusions

This rapid, convenient atomic-absorption spectrophotometric method for the determination of tellurium in the range 0·005–0·080 per cent. in plain and leaded free-cutting steels gives results that are in close agreement with those given by the existing turbidimetric method and good reproducibility. Inter-element interference is very slight and the method is also applicable to stainless steels.

Six determinations can be completed in about 1·5 h but this time is proportionately reduced by incorporating the procedure in the composite scheme, which includes lead, to considerable practical advantage.

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References

1. Marcec, M. V., Kinson, K., and Belcher, C. B., *Analytica Chim. Acta*, 1968, **41**, 447.
2. Inland Steel Co., U.S.A., personal communication, 7th March, 1962.
3. Chakrabarti, C. L., *Analytica Chim. Acta*, 1967, **39**, 293.
4. Harrison, T. S., Foster, W. W., and Cobb, W. D., *Metallurgia Metal Forming*, 1973, **40**, 361.

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Colorimetric Determination of Phosphorus in Silicates Following Fusion with Lithium Metaborate

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The determination of phosphorus in silicate materials by use of a colorimetric technique following fusion with lithium metaborate is described. The method is rapid and the results obtained compare favourably with the accepted values for standard rock and mineral samples and also with gravimetric results. Reliable values of up to 1·75 per cent. for the phosphorus(V) oxide content have been obtained by this method.

Although phosphorus is rarely present in amounts greater than those of minor constituents, it is nearly always determined in analyses of silicate rocks. Phosphorus content is an important component in chemical calculations involving rock analyses and is significant in petrological models devised to explain the origin and subsequent history of basaltic magmas. Basic silica-undersaturated rocks, such as trachybasalts and basanitoids, may contain several per cent. *m/m* of phosphorus(V) oxide, whereas in granitic rocks and in minerals the content is much lower and often is in the parts per million range. The wide range of phosphorus(V) oxide contents has been a factor in the use of various colorimetric methods that have been proposed for its determination.[1–3]

The introduction of the lithium metaborate - nitric acid solution technique[4] has led to the rapid determination of the major and minor constituents in rocks and minerals by atomic-absorption spectrophotometry.[5,6] The need for a reliable and rapid determination of phosphorus has resulted in the development of a colorimetric procedure using the same sample solution as that analysed for the major constituents.

The most sensitive methods for determining phosphorus have involved the reduction of molybdenum(VI) to molybdenum(V) and determination of the intensity of the colour of the molybdenum or heteropoly blue formed by this species. Ingamells[7] proposed a method for the rapid colorimetric determination of phosphorus in the presence of silica following dissolution of the sample using a lithium metaborate - nitric acid solution technique. We obtained erratic results with this method, however, and attempts to improve it by removal of the silica also proved unsuccessful.

The method of Fogg and Wilkinson[8] was found to be reliable for the determination of phosphorus and readily adaptable to a sample solution obtained by the lithium metaborate - nitric acid solution technique, but it is necessary to treat the sample solution with hydrofluoric and sulphuric acids in order to remove the silica.

Experimental

Reagents

Lithium metaborate. Anhydrous $LiBO_2$.
Nitric acid, 4 per cent. V/V.
4-Nitrophenol solution, 0·2 per cent. m/V.
Sulphuric acid, 1 N.
Sodium hydroxide solution, 1 N.
Ascorbic acid.
Ammonium molybdate reagent. Dissolve 10 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ in 75 ml of water. Heat in order to effect dissolution and then allow to cool to room temperature. Filter into a 100-ml calibrated flask and dilute to volume. Add the solution with stirring to 300 ml of 50 per cent. *V/V* sulphuric acid contained in a polyethylene bottle. Cool the resulting solution to room temperature. This reagent is stable for approximately 1 month but should be discarded either on the formation of a precipitate or on the development of a blue colour.
Standard phosphate solution. Dissolve 0·7669 g of potassium dihydrogen orthophosphate in distilled water and dilute to 1 l.

1 ml of solution ≡ 400 μg of phosphorus(V) oxide.

Dilute standard phosphate solution. Dilute 25·0 ml of the standard phosphate solution to 1 l.

1 ml of solution ≡ 10 μg of phosphorus(V) oxide.

Instrumentation

A Bausch and Lomb Spectronic 70-6 spectrophotometer and 20-mm absorbance cells were used for all determinations.

Procedure

The lithium metaborate - nitric acid solution technique has been reported previously[4] but slight modifications have since been incorporated. The method is included here for completeness.

A 200-mg amount of sample, ground to pass a 200-mesh sieve (ASTM), is mixed with 800 mg of anhydrous lithium metaborate. The mixture is transferred to a pre-ignited graphite fusion crucible, which is placed in a muffle furnace at 1050 °C for 10 min. While molten, the fused material is poured into a polypropylene beaker containing 80·0 ml of 4 per cent. *V/V* nitric acid. The beaker is covered and placed on a magnetic stirrer until the sample is completely dissolved (usually 5–10 min). This sample solution is used without further dilution. One millilitre of this solution is equivalent to 0·0025 g of sample.

A 5·00-ml aliquot (0·0125 g of sample) of the solution is placed in a 55-ml platinum or PTFE dish. Five millilitres of hydrofluoric acid and 5 drops of 50 per cent. *V/V* sulphuric acid are added. The dish is placed on a hot-plate and the solution heated slowly to fumes of sulphur(VI) oxide. The fuming is continued nearly to dryness. After cooling, the residue is moistened with water and a further 5 drops of 50 per cent. *V/V* sulphuric acid are added, the solution being taken to dryness. The dish is again cooled and 5 drops of 50 per cent. *V/V* sulphuric acid and 5 ml of water are added. The dish is heated in order to effect complete dissolution and the solution is then transferred to a 100-ml beaker with water, washings from the dish being added to the beaker.

The acidity is adjusted by adding 2 drops of 0·2 per cent. *m/V* 4-nitrophenol indicator solution followed by the dropwise addition of 1 N sodium hydroxide solution until a deep yellow colour is obtained. Sulphuric acid (1 N) is added dropwise until the solution is colourless. After diluting the solution to 40 ml ammonium molybdate reagent (4·0 ml) is added, followed by 0·2 g of ascorbic acid. The solution is heated to boiling, boiled for 1 min and then cooled to room temperature before transferring it to a 50-ml calibrated flask and diluting to volume. The absorbance of the solution at 810 nm is measured, using a 20-mm cell, and with Specpure silica being carried through the entire procedure as a blank solution. The phosphorus(V) oxide concentrations are determined from a calibration graph prepared by using the dilute standard phosphate solution.

Up to 0·40 per cent. *m/m* of phosphorus(V) oxide can be determined using a 50-ml calibrated flask as described. Higher phosphorus concentrations were determined by using larger calibrated flasks and adjusting the blank value accordingly.

Preparation of Calibration Graph

Into a series of 100-ml beakers place 0, 0·5, 1·0, 2·0, 3·0, 4·0 and 5·0 ml of the dilute standard phosphate solution. Follow the same steps for developing the colour as described under Procedure, starting with the paragraph "The acidity is adjusted by . . . ."

Discussion

The method chosen for the colour development is essentially that of Fogg and Wilkinson,[8] who applied their procedure to the determination of phosphorus in boiler water, effluents and salt deposits. The colour development takes place rapidly on boiling the solution for 1 min and is stable for an indefinite period. Silicon and arsenic are the only elements that interfere, both elements reacting to form the molybdenum-blue colour under the same experimental conditions. Although use of this procedure[8] appears to overcome the interference by silica, attempts to determine the phosphorus in the presence of silica in lithium metaborate - nitric acid solution were unsuccessful. The developed colour faded immediately. The removal of silica by volatilisation with hydrofluoric acid and sulphuric acid overcomes this problem.

TABLE I

COMPARISON OF RESULTS OBTAINED FOR PHOSPHORUS(V) OXIDE CONTENT FOR VARIOUS STANDARDS BY PROPOSED METHOD WITH ACCEPTED VALUES

		Phosphorus(V) oxide content, per cent.		
			Accepted value	
Source	Material	Designation	This work	Value	Reference
USGS*	Granite	G-1	0·069	0·09	9
	Diabase	W-1	0·129	0·14	9
	Granite	G-2	0·133	0·14	10
	Granodiorite	GSP-1	0·286	0·28	10
	Basalt	BCR-1	0·363	0·36	10
	Andesite	AGV-1	0·494	0·49	10
CRPG†	Granite	GH	0·007	0·01	11
	Granite	GA	0·121	0·12	11
	Basalt	BR	1·05	1·04	11
CSRM‡	Syenite	S-1	0·212	0·22	12

* United States Geological Survey, Reston, Va., U.S.A.
† Centre de Recherches Petrographiques et Geochimiques, Nancy, France.
‡ Canadian Standard Reference Materials, Mines Branch, Ottawa, Ontario, Canada.

Arsenic is not usually found in significant amounts in most rock and mineral samples. Arsenic present as arsenate interferes, whereas arsenite does not. Reduction of arsenate to arsenite should overcome this interference, although this has not been attempted.

The proposed method permits the determination of phosphorus on the same sample solution as that used for the determination of the major elements and the phosphorus is easily and rapidly determined. The method has been applied to a wide range of materials and no problems have been encountered.

TABLE II

COMPARISON OF RESULTS OBTAINED FOR PHOSPHORUS(V) OXIDE CONTENT BY THE PROPOSED METHOD AND BY GRAVIMETRIC METHODS

	Phosphorus(V) oxide content, per cent.	
Material	Proposed method	Gravimetric method*
Granite Gneiss	0·100	0·097
		0·108
Granite Aplite	0·036	0·034
Granite Aplite	0·042	0·036
Granodiorite Gneiss	0·093	0·092
		0·098
Granite Aplite	0·048	0·053
		0·043
		0·044
Granite	0·022	0·023
		0·029
		0·023
Granite Gneiss	0·091	0·096
		0·092
Amphibolite	0·247	0·239
Trachybasalt	0·469	0·451
		0·455
Trachybasalt	0·767	0·765
		0·755
Basanitoid	0·797	0·798
		0·799
Trachybasalt	1·49	1·52
		1·51
Trachybasalt	1·75	1·76
		1·77

* Gravimetric determinations by S. S. Goldich, Geology Department, Northern Illinois University, DeKalb, Ill., U.S.A.

The results obtained for phosphorus(V) oxide in some standard rock samples are compared with the accepted values in Table I. Agreement with the accepted values is within 0·01 per cent. in all instances, with the exception of Granite G-1, for which the difference is 0·02 per cent. However, an independent gravimetric determination of phosphorus on a sample from the same bottle as that used in the colorimetric determination yielded a value of 0·072 per cent. of phosphorus(V) oxide, which is in agreement with the value obtained (0·069 per cent.) by the proposed colorimetric method. Examination of the data given by Fleischer[9] shows many other values of about 0·07 per cent. for colorimetric determinations of phosphorus.

A number of samples were independently analysed by two different gravimetric methods and also by the proposed colorimetric procedure. The gravimetric methods for these separate determinations were: (1), a single precipitation and weighing as ammonium molybdophosphate; and (2), a preliminary precipitation as ammonium molybdophosphate followed by precipitation as ammonium magnesium phosphate and ignition to give magnesium pyrophosphate ($Mg_2P_2O_7$). These results are compared in Table II and indicate the range of the method. It is possible to extend the range to include at least twice the highest value given in the table (1·75 per cent.) by a combination of increasing the volumes and selecting smaller absorbance cells.

Table III contains values for phosphorus(V) oxide in a number of new standard rock and mineral samples for which very few or tentative results have been published.

TABLE III

VALUES OBTAINED BY THE PROPOSED METHOD FOR PHOSPHORUS(V) OXIDE CONTENT OF NEW STANDARD SAMPLES

Source	Material	Designation	Phosphorus(V) oxide content, per cent.
CRPG	Biotite	Mica-Fe	0·420
	Phlogopite	Mica-Mg	0·002
	Diorite	DR-N	0·249
	Serpentine	UB-N	0·010
	Disthene	DT-N	0·087
	Granite	GS-N	0·282
	Bauxite	BX-N	0·142
	Feldspar	FK-N	0·009
CSRM	Gabbro	MRG-1	0·054
	Syenite	SY-2	0·441
	Syenite	SY-3	0·560
NIM*	Granite	NIM-G	0·012
	Dunite	NIM-D	0·020
	Norite	NIM-N	0·015
	Pyroxenite	NIM-P	0·024
	Lujavrite	NIM-L	0·049
	Syenite	NIM-S	0·119

* National Institute for Metallurgy, Aukland Park, South Africa.

Mean values, standard deviations and coefficients of variation for four USGS standards are listed in Table IV. These values were obtained from individual determinations carried out routinely over a period of 2 years. The data indicate that the proposed method is capable of good reproducibility on a routine basis. The mean values reported in Table IV are slightly different from the values reported in Table I, which were obtained from single determinations carried out as a special project.

TABLE IV

PRECISION DATA

Sample	Mean phosphorus(V) oxide content, per cent.	Standard deviation, per cent.	Number of determinations	Coefficient of variation, per cent.
W-1	0·131	0·0044	7	3·36
G-2	0·133	0·0043	12	3·22
BCR-1	0·360	0·0036	10	1·00
AGV-1	0·496	0·0105	13	2·12

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References

1. Baadsgaard, H., and Sandell, E. B., *Analytica Chim. Acta*, 1954, **11**, 183.
2. Maxwell, J. A., "Rock and Mineral Analysis," Interscience Publishers, New York, 1968, p. 394.
3. Riley, J. P., *Analytica Chim. Acta*, 1958, **19**, 425.
4. Suhr, N. H., and Ingamells, C. O., *Analyt. Chem.*, 1966, **38**, 730.
5. Medlin, J. H., Suhr, N. H., and Bodkin, J. B., *Atom. Absorption Newsl.*, 1969, **8**, 25.
6. Medlin, J. H., Suhr, N. H., and Bodkin, J. B., *Chem. Geol.*, 1970, **6**, 143.
7. Ingamells, C. O., *Analyt. Chem.*, 1966, **38**, 1228.
8. Fogg, D. N., and Wilkinson, N. T., *Analyst*, 1958, **83**, 406.
9. Fleischer, M., *Geochim. Cosmochim. Acta*, 1969, **33**, 65.
10. Flanagan, F. J., *Geochim. Cosmochim. Acta*, 1973, **37**, 1189.
11. de la Roche, H., and Govindaraju, K., *Méth. Phys. Analyse*, 1971, **7**, 314.
12. Sine, N. M., Taylor, W. O., Webber, G. R., and Lewis, C. L., *Geochim. Cosmochim. Acta*, 1969, **33**, 121.

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Determination of Benzoic and Sorbic Acids in Orange Juice

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A procedure has been devised for the separate determination of benzoic and sorbic acids in orange juice. It is based on the steam distillation of these preservatives from an acidified juice into an alkaline trap. The distillate is divided into two portions, each of which is treated with potassium dichromate and sulphuric acid, but under different conditions. Drastic oxidation leads to the destruction of sorbic acid and, after re-distillation, allows the determination of benzoic acid at 225 nm (with no interference from sorbic acid). Milder oxidation selectively converts sorbic acid into malonaldehyde, and the latter is subsequently converted into a coloured compound and determined at 532 nm (with no interference from benzoic acid).

Recoveries of both preservatives from orange juice were satisfactory.

A direct spectrophotometric method of analysis for the determination of both sorbic and benzoic acids has been described by various investigators.[1–3] Generally, the methods are based on measurements of light absorption at two wavelengths (approximately 225 and 255 nm) of either an ether extract or a distillate containing the above acids. The procedure is fairly rapid, but its accuracy is rather poor. In the region of 225 nm (the wavelength of maximum absorbance of benzoic acid) the spectrum given by a sorbate has a steep slope and its absorbance is high in comparison with that of a benzoate. This coincidence leads to erroneous readings when both benzoate and sorbate are present in the same solution. In addition, determinations in the ultraviolet region may yield results that are too high, owing to the presence of various light-absorbing substances from the examined food product in the extract or the distillate.

Several workers reported better results by using a blank which they derived from a sample without preservatives.[4,5] Such a blank is not usually obtainable, because with a commercial sample it is difficult to have knowledge of all of its ingredients; the presence and concentrations of these ingredients will depend also on technological processes and/or on conditions of storage. Another group of workers[6] used a blank that was obtained by distillation of a neutralised sample (from which the investigated preservatives will not volatilise). Such a blank has drawbacks, however, as it does not reflect those interfering components which will steam distil from an acidified sample.

Several investigators[7–10] successfully determined sorbic acid in various food products by the method of Schmidt,[7] which is based on the colorimetric measurement of the condensation product of malonaldehyde (a sorbic acid derivative) with thiobarbituric acid (TBA).

The proposed procedure for the determination of benzoic and sorbic acids in orange juice (see Fig. 1) calls for the determination of each of these preservatives under conditions that render the solution devoid of interferences caused by the other preservative and by interfering substances in the juice itself. Benzoic and sorbic acids are distilled off from an acidified juice. A portion of the distillate is subjected to oxidation with potassium dichromate under drastic conditions. Sorbic acid and volatile compounds from the juice are destroyed by this process and the unaffected benzoic acid is re-distilled and determined at 225 nm. The remainder of the distillate is subjected to oxidation under much milder conditions (also with potassium dichromate). Malonaldehyde is formed and subsequently converted into a highly coloured component; the latter is determined at 532 nm,[7] while neither benzoic acid nor volatile compounds from the juice interfere.

Experimental

Apparatus

Distillation apparatus. See Fig. 2.

Spectrophotometer. Visible and ultraviolet ranges.

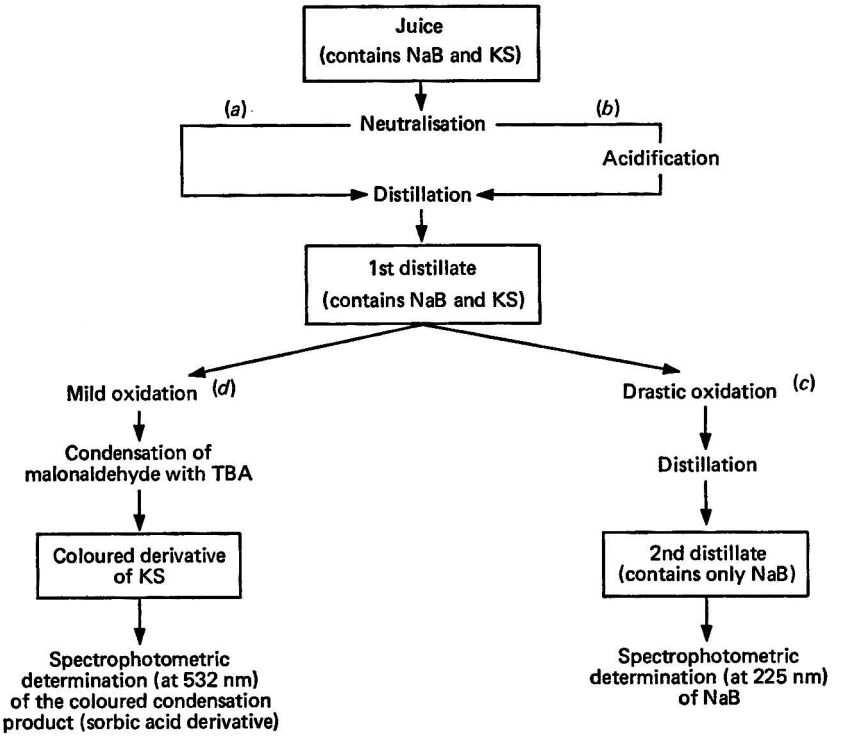

Fig. 1. Scheme for the determination of sodium benzoate (NaB) and potassium sorbate (KS) in orange juice. (*a*), Pathway of the blank distillate (blank 1) (see Experimental); (*b*), pathway of the main distillate (and of blank 2) (see Experimental); (*c*), oxidation with 0·2 N $K_2Cr_2O_7$ and 4 N H_2SO_4. Sorbic acid and volatiles from the juice are oxidised to substances that do not absorb at 225 nm. (*d*), Oxidation with 0·01 N $K_2Cr_2O_7$ and 0·3 N H_2SO_4. Malonaldehyde is produced by oxidation of sorbic acid.

Reagents

Analytical-reagent grade reagents are to be used throughout the determination.

Phenolphthalein solution. Dissolve 1 g of phenolphthalein in 100 ml of ethanol (95 per cent.).

Sodium sulphate, anhydrous, granular.

Orthophosphoric acid, 85 per cent.

Sodium hydroxide solution, 0·1 N. Dissolve 2 g of sodium hydroxide in 500 ml of water.

Sodium hydroxide solution, 0·01 N. Dilute 10 ml of 0·1 N sodium hydroxide solution to 100 ml with water.

Potassium dichromate solution, 0·2 N. Dissolve 4·9 g of potassium dichromate in water and dilute to 500 ml with water.

Potassium dichromate solution, 0·01 N. Dilute 5 ml of 0·2 N potassium dichromate solution to 100 ml with water.

Sulphuric acid, 4 N. Dilute 66·5 ml of sulphuric acid (concentrated) to 500 ml with water.

Sulphuric acid, 0·3 N. Dilute 7·5 ml of 4 N sulphuric acid to 100 ml with water.

Thiobarbituric acid solution, 0·5 *per cent.* Dissolve 0·5 g of thiobarbituric acid in 20 ml of water plus 10 ml of 1 N sodium hydroxide solution, add 11 ml of 1 N hydrochloric acid and dilute the mixture to 100 ml with water. Prepare the solution fresh daily.

Sodium benzoate stock solution, 100 *p.p.m.* Dissolve 100 mg of sodium benzoate (which has has previously been dried at 105 °C for 2 h) in water in a 1000-ml calibrated flask. Add 100 ml of 0·1 N sodium hydroxide solution and dilute to 1000 ml with water.

Sodium benzoate working solution, 20 *p.p.m.* Dilute 20 ml of the stock solution to 100 ml with water.

Potassium sorbate stock solution, 100 *p.p.m.* Dissolve 100 mg of potassium sorbate (which has previously been dried at 105 °C for 2 h) in water in a 1000-ml calibrated flask. Add 100 ml of 0·1 N sodium hydroxide solution and dilute to 1000 ml with water.

Potassium sorbate working solution, 2 *p.p.m.* Dilute 2 ml of the stock solution of potassium sorbate to 100 ml with water.

Preliminary Experiments

Several experiments were performed on juice containing no preservatives. In these experiments blank 1 was prepared from a neutralised juice according to the method described under *Preparation of blank distillate* (*blank* 1) and blank 2 from an acidified juice according to the method described under *Preparation of main distillate.*

Procedure

Preliminary operations

Place an accurately weighed sample of about 5 g of juice into a 100-ml calibrated flask. Add 50 ml of water to the juice and neutralise the sample with 0·1 N sodium hydroxide solution until the appearance of a pink colour in the presence of phenolphthalein. Dilute the solution to 100 ml with water. (In our experiments distilled water or freshly pressed and pasteurised orange juice was used for preparation of the model solutions of benzoic and sorbic acids.)

Preparation of main distillate

Pipette 20 ml of the neutralised sample solution (above) or a portion containing about 200–1000 μg of sodium benzoate and about 100–500 μg of potassium sorbate into a 250-ml distillation flask (A, Fig. 2). Next add 1 ml of orthophosphoric acid, 20 g of anhydrous sodium sulphate and about 30 ml of water and heat the solution vigorously over a gas flame. Adjust the rate of heating so as to obtain 35 ml of distillate in about 10 min and collect the distillate in a 50-ml calibrated flask (C, Fig. 2) that contains 5 ml of 0·1 N sodium hydroxide solution. Then rinse the condenser (B, Fig. 2) and dilute the distillate to 50 ml with water.

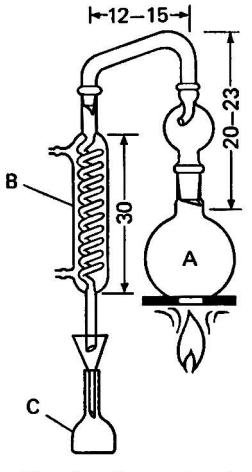

Fig. 2. Apparatus for distillation of benzoic and sorbic acids.[11] A, distillation flask, 250 ml; B, condenser; C, calibrated flask, 50 ml. Dimensions are in centimetres.

Preparation of blank distillate (*blank* 1)

Repeat the above procedure using the neutralised juice without addition of orthophosphoric acid.

Determination of benzoic acid

Preparation of standard graph. Transfer 1-, 2-, 3-, 4- and 5-ml portions of the standard sodium benzoate working solution into 10-ml calibrated flasks, each containing 1 ml of 0·1 N sodium hydroxide solution. Dilute the solution in each flask to the mark with distilled water and mix. Read the absorbance of each solution at 225 nm against 0·01 N sodium hydroxide solution and plot a graph of the concentration of sodium benzoate (in p.p.m.) *versus* absorbance (at 225 nm).

Determination in distillate. Place 25 ml of each distillate (main and blank) in separate distillation flasks of 250-ml capacity. Add 25 ml of 0·2 N potassium dichromate solution and 6·5 ml of 4 N sulphuric acid to each. Heat in a boiling water bath for exactly 10 min, then cool and proceed as described under *Preparation of main distillate*, commencing at "Next add 1 ml of orthophosphoric acid . . . ."

Measure the absorbance values of the two solutions at 225 nm against 0·01 N sodium hydroxide solution. Subtract the absorbance of the blank from that found for the main solution, then from the standard graph find the concentration of sodium benzoate corresponding to the corrected absorbance. Calculate the content of sodium benzoate in the juice as follows:

$$\text{Sodium benzoate, p.p.m.} = 100\ M/W$$

where M p.p.m. is the concentration of sodium benzoate in the portion of distillate (corresponding to the value found from the calibration graph), W g is the mass of sample taken for distillation and 100 is the dilution factor.

Determination of sorbic acid

Preparation of standard graph. Transfer 0-, 1-, 2-, 3-, 4- and 5-ml portions of the working solution of potassium sorbate into separate 10-ml calibrated flasks and dilute them to 5 ml with water. To each flask add 1 ml of 0·01 N potassium dichromate solution and 1 ml of 0·3 N sulphuric acid and mix. Heat the solutions in a boiling water bath for exactly 5 min, add 2 ml of thiobarbituric acid solution and heat them again in a boiling water bath for an additional 10 min (exactly). Cool and dilute the solutions to 10 ml with water, then read their absorbance values at 532 nm against a reagent blank. Plot a graph of the concentration of potassium sorbate (in p.p.m.) *versus* absorbance (at 532 nm).

Determination in distillate. Place 1 ml of each distillate (main and blank) in separate 10-ml calibrated flasks. Dilute each to 5 ml with water and proceed as described under *Preparation of standard graph*, commencing at "add 1 ml of 0·01 N potassium dichromate . . . ." Subtract the absorbance of the blank from that found for the main solution and from the standard graph find the concentration of potassium sorbate corresponding to the corrected absorbance. Calculate the potassium sorbate content in the juice as follows:

$$\text{Potassium sorbate, p.p.m.} = 500\ M/W$$

where M p.p.m. is the concentration of potassium sorbate in the portion of distillate (corresponding to the value found from the calibration graph), W g is the mass of sample taken for distillation and 500 is the dilution factor.

Results and Discussion

It can be seen from Table I that when the single-distillation procedure[2] was used, blank 1 (a distillate from neutralised juice) did not have contributions to the absorbance of light in the ultraviolet region from some of the volatile substances in the juice which were present in the distillate from acidified juice. Higher absorptions were always recorded for blank 2 (a distillate from acidified juice of the same origin, but without preservatives). In fact, the latter blank should be used in the determinations of benzoic and sorbic acids described, but this is, of course, impossible as no samples of juice without preservatives of the same origin and with the same history of processing and storage will be available at the time of determination of the preservatives in a commercial sample. An analyst operating according to the procedure of Monselise[2] is compelled to use blank 1 and will therefore obtain results that are too high (owing to the lower absorbance of that blank, see above).

The proposed method overcomes the difficulty by concluding the procedure in two stages

TABLE I

ABSORBANCE OF BLANKS 1 AND 2 OBTAINED FROM ORANGE JUICE WITH NO PRESERVATIVES

Determinations were made in triplicate.

Procedure	Mass of juice taken for distillation/ g	Sample A		Sample B	
		Blank 1*	Blank 2†	Blank 1*	Blank 2†
$\lambda = 225$ *nm*—					
Single distillation[2]	5	0·114	0·204	0·169	0·191
Proposed	5	0·088	0·096	0·126	0·130
Single distillation[2]	1	0·040	0·097	0·034	0·105
Proposed	1	0·049	0·054	0·033	0·029
$\lambda = 532$ *nm*—					
Proposed	5	0·000	0·008	0·012	0·017
Proposed	1	0·000	0·000	0·006	0·006

* From neutralised juice, see Preliminary experiments.
† From acidified juice, see Preliminary experiments.

(see Fig. 1). (*i*) In one portion of the distillate from the acidified juice, interfering volatile compounds are eliminated by drastic oxidation; this is followed by re-distillation of benzoic acid. Sorbic acid is also destroyed during the oxidation, which is advantageous as the benzoic acid in the re-distilled liquid can be determined without interference from the other preservative. (*ii*) In another portion of the distillate from the acidified juice, sorbic acid is converted first into malonaldehyde and then into a coloured derivative; the absorbance of the latter is read in the visible-light region, at a wavelength at which the presence of neither the volatile compounds from the juice nor benzoic acid can interfere.

It has been shown (Table I) that in the proposed procedure the respective readings at 225 nm (determination of benzoic acid) and 532 nm (determination of sorbic acid) were virtually the same for blanks from either neutralised or acidified juice with no preservatives. Linear graphs of absorbance *versus* concentration were obtained for sodium benzoate and potassium sorbate up to 10 p.p.m. (with absorbances of up to 0·6) and 1 p.p.m. (with absorbances of up to 0·3), respectively.

TABLE II

RECOVERY OF SODIUM BENZOATE AND POTASSIUM SORBATE FROM MODEL SOLUTIONS IN WATER OR IN ORANGE JUICE*

Determinations were made in duplicate.

Solution	Concentration, p.p.m.	Mass of sample taken for distillation/ g	Recovery of each component, per cent.
In water—			
Sodium benzoate	40	5	97
Potassium sorbate	40		98
Sodium benzoate	400	1	97
Potassium sorbate	400		96
In orange juice—			
Sodium benzoate	40	5	99
Potassium sorbate	40		104
Sodium benzoate	400	2	96
Potassium sorbate	150		109
Sodium benzoate	300	1	98
Potassium sorbate	300		99
Sodium benzoate	500	1	104
Potassium sorbate	500		92
Sodium benzoate	1000	1	92
Potassium sorbate	500		97

* In all the experiments blank 1 was used.

The results obtained for the determination of benzoate and sorbate in model solutions are summarised in Table II. The distillation (or re-distillation) time required for recovery of the preservatives was 10 min. The recoveries of both preservatives were satisfactory (92–104 per cent. for benzoate and 92–109 per cent. for sorbate).

In conclusion, the proposed method facilitates the determination of benzoic and sorbic acids with no interference from each other or from light-absorbing (in the ultraviolet region) volatile compounds in the juice. While being more elaborate than the other methods,[1–3] it remains fairly simple, as each step can be easily and rapidly carried out. The results obtained by use of this method are satisfactory and reproducible. The method was tested with orange juice but the authors believe that it can also be used for other fruit and vegetable juices.

References

1. Lorenzen, W., and Sieh, R., *Z. Lebensmittelunters. u. -Forsch.*, 1962, **118**, 223.
2. Monselise, J. J., *Israel J. Technol.*, 1969, **7**, 263.
3. Gantenbein, W. M., and Karasz, A. B., *J. Ass. Off. Analyt. Chem.*, 1969, **52**, 738.
4. Luckman, F. H., and Melnick, D., *Fd Res.*, 1955, **20**, 649.
5. English, E., *Analyst*, 1959, **84**, 465.
6. Monselise, J. J., Friedman, Y., and Fathi, R., *Israel J. Technol.*, 1970, **8**, 437.
7. Schmidt, H., *Z. Analyt. Chem.*, 1960, **178**, 173.
8. Nury, F. S., and Bolin, H. R., *J. Fd Sci.*, 1962, **27**, 370.
9. Wilamowski, G., *J. Ass. Off. Analyt. Chem.*, 1971, **54**, 663.
10. Caputi, A., and Slinkard, K., *J. Ass. Off. Analyt. Chem.*, 1975, **58**, 133.
11. Israeli Standard No. 862, 1973.

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Application of the Tubular Graphite Electrode in the Measurement of Reaction Kinetics: Development of an Automatic Technique

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The unique feature of the tubular electrode, that it permits continuous analysis of an electrolyte solution passing through it, has been made use of in developing an automatic voltammetric technique for studying the kinetics of reactions involving one or more electro-active reactants or products. The technique provides for automatic dilution of the reaction mixture with the supporting electrolyte in order to bring the concentrations within the voltammetric range, automatic freezing of the reaction when required, automatic stirring of the reaction mixture solution and, especially, automatic recording of the current - time graph, from which the kinetics of the reactions can easily be studied.

The technique has been standardised by studying voltammetrically the reaction kinetics of the oxidation of potassium iodide with hydrogen peroxide in aqueous acidic solutions and comparing the data with those obtained titrimetrically. The results appear to show that the automatic technique has a high dependability. Encouraging results have been obtained in the study of the reaction kinetics of the oxidation of potassium hexacyanoferrate(II) with hydrogen peroxide and of sodium formate with potassium permanganate in acidic media.

The tubular graphite electrode[1–5] can be conveniently used for the continuous analysis of an electro-active solution passing through it at a given flow-rate. It was thought of interest to use this unique property in the study of kinetics of reactions involving one or more electro-active reactants or products, and particularly to develop and standardise an automatic technique for this purpose.

It is proposed that the reaction should proceed in a vessel such as a gravity feed with constant head with the reactants at any desired concentration. The reaction mixture coming out of the vessel is then diluted to the concentrations required for the voltammetric measurements by mixing it with a pre-chilled solution of the supporting electrolyte before it passes through the electrode. The dilution process also has the essential function of freezing the reaction at that instant.

Experimental

Apparatus

The complete assembly, designed and fabricated for the present work, is shown in Fig. 1 (*a*). The main component of the assembly is the mixer, which is required for the instantaneous and thorough mixing of the reaction mixture with the supporting electrolyte solution. The mixer is shown separately in Fig. 1 (*b*). It consists of a cylindrical glass tube about 5 cm in diameter and about 13 cm in length, with a slanting plate, P, across the centre and a delivery tube, D, near the top. The plate has a hole of about 7 mm diameter towards its lower end.

The reaction under investigation is allowed to proceed in the reservoir of the double-jacketed gravity feed G_1 and the supporting electrolyte solution is placed in the reservoir of the gravity feed G_2, which is surrounded by an ice jacket. The reaction mixture solution, flowing out of the jet, J, at a very small flow-rate that is controlled by adjusting the length of the tube T_1 of the gravity feed G_1, is picked up by the pre-cooled supporting electrolyte solution, which flows from the delivery tube, D, at a much higher rate (about 100 times higher). This latter flow-rate is controlled by the length of the tube T_2 of the gravity feed G_2 and also by the height of G_2 with respect to the mixer. The two solutions are mixed fairly well in this way and the reaction is simultaneously brought almost to a standstill. Greater opportunity for mixing is provided as the solutions pass through the mixer, as illustrated in Fig. 1. It is

expected, therefore, that the solution which comes out of the mixer through the side tube, S, is composed of a completely uniform mixture of the two solutions. As only a limited amount of the solution is to be passed through the tubular electrode, a major portion of the solution is by-passed through the overflow tube, Q, which is fixed in such a way as to allow only a negligible retention of the solution [Fig. 1 (*b*)].

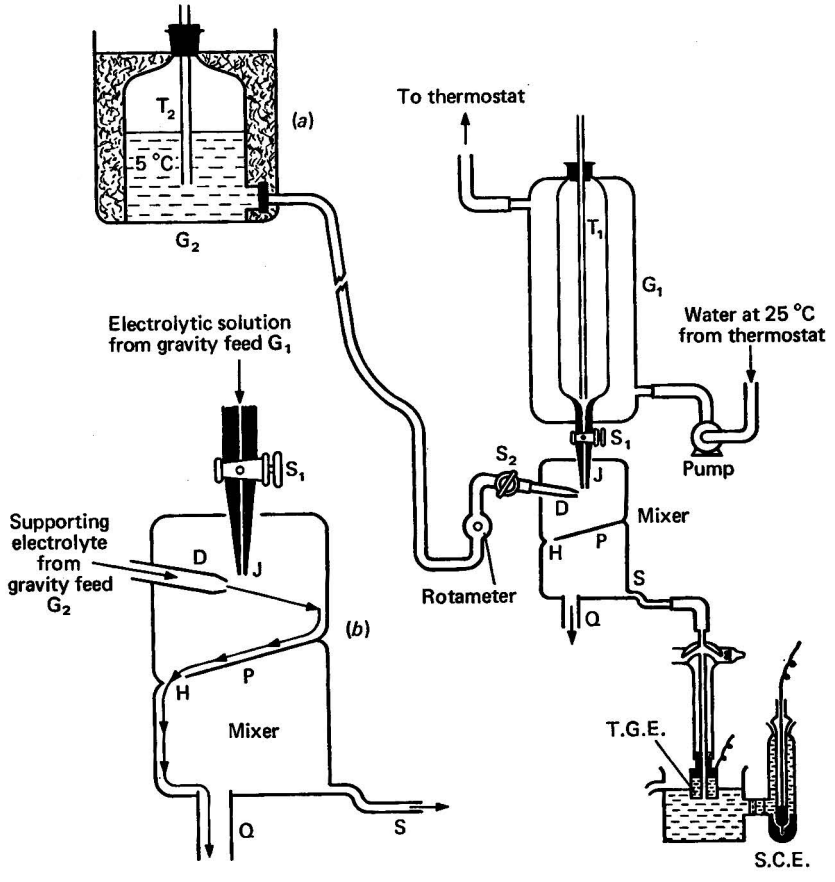

Fig. 1. (*a*), Apparatus designed for use in the study of reaction kinetics by automatic methods. (*b*), Mixer on an enlarged scale. Lettered components are identified in the text. T.G.E. = tubular graphite electrode.

The efficiency of the mixer in bringing about instantaneous and uniform mixing of the solutions was tested by mixing a solution of methylene blue from gravity feed G_1 with a large excess of water from gravity feed G_2, collecting portions of the mixture coming out of the side tube, S, at different intervals of time and subjecting them to colorimetric analysis. The fact that the concentration of dye solution in the first portion was found to be exactly the same as that in subsequent portions, shows that the mixer is capable of effecting a satisfactory mixing of the two solutions.

Selection of the Reaction for Kinetic Measurements

The oxidation in acidic medium of potassium iodide by hydrogen peroxide

$$2KI + H_2O_2 \rightarrow 2KOH + I_2$$

was considered to be a suitable reaction with which to study the kinetics by voltammetric methods, as the concentration of one of the reactants, namely with the iodide ions, in the

reaction mixture could easily be determined by carrying out its electro-oxidation at the tubular graphite electrode.

Blank Experiment

A blank experiment was run by placing 0·1 M sulphuric acid that was 10^{-2} M in potassium iodide in the gravity feed G_1 and 0·1 M sulphuric acid in the gravity feed G_2. The iodide solution was maintained at 25 °C by circulating water from the thermostat at 25 °C through the outside jacket of the gravity feed G_1. The supporting electrolyte solution was cooled to 5 °C by surrounding the gravity feed G_2 with crushed ice.

The flow-rate of the iodide solution was adjusted to 5 ml min^{-1} by altering the length of the tube T_1 dipping into the iodide solution. Similarly, the flow-rate of the supporting electrolyte solution (measured by the rotameter, R) was adjusted to 495 ml min^{-1} by altering the height of the gravity feed G_2 and the length of the tube T_2 dipping into the solution. The iodide solution coming out of the mixer and then passing through the electrode had a concentration of 10^{-4} M. A complete voltammogram for the oxidation of iodide ions was then scanned by following the procedure described earlier.[1] The flow-rate of the solution passing through the electrode was measured accurately and the limiting current at a flow-rate of 10·8 ml min^{-1} was found to be 19·5 μA.

Voltammograms were also run for mixtures of potassium iodide and iodine solutions of appropriate concentrations. In each instance, an extremely well defined voltammogram with a well defined diffusion plateau was obtained, irrespective of the presence or absence of iodine in solution. The limiting current was found to be directly proportional to the concentration of the iodide ions.

Automatic Kinetic Measurements

The following procedure was adopted for the study of the kinetics of the reaction selected. Equal volumes of 0·02 M potassium iodide and 0·02 M hydrogen peroxide solutions, both kept thermostatically at 25 °C, were mixed in a beaker and at the same instant the chart drive of the polarograph was switched to the "on" position. The mixture was immediately transferred to the reservoir of the gravity feed G_1 and the tube T_1 introduced into the vessel. Water at 25 °C was circulated around the reservoir continuously. The stopcock S_2 of the gravity feed G_2 was kept open and the supporting electrolyte solution, 0·1 M sulphuric acid cooled to 5 °C, was kept flowing through the mixer at a constant rate of 495 ml min^{-1}. The stopcock S_1 of the gravity feed G_1 was then opened and concurrently (at time t_1) a mark was put on the time axis of the recorder chart. As the retention of the solution by the capillary jet below the stopcock S_1 was extremely small, the reaction mixture, flowing at a rate of 5 ml min^{-1}, took only a negligible time to clear the jet. As soon as the solution came out of the jet, J, it met the current of pre-chilled electrolyte solution, which resulted in the requisite dilution as well as in the freezing of the reaction at that instant. After passing through the mixer the mixed solution entered the tubular graphite electrode, which was maintained at a potential of 0·75 V *versus* S.C.E. This potential lies well within the range for the diffusion plateau of the voltammogram obtained for the oxidation of iodide ions. As soon as the reaction mixture passed through the electrode a limiting current proportional to the concentration of the iodide ions in the mixture was recorded on the chart. It is evident that the limiting current thus recorded corresponded to that concentration of iodide ions present in the solution at the instant of opening the stopcock S_1. With the progress of the reaction, there was a continuous decrease in the concentration of the iodide ions and a corresponding decrease in the magnitude of the limiting current, recorded automatically on the recorder chart. Thus a current - time graph (which is equivalent to a concentration - time graph) was recorded automatically during the oxidation of potassium iodide by hydrogen peroxide. This graph is shown in Fig. 2.

The horizontal portion, from zero to A of the graph represents the residual current when only the supporting electrolyte solution from the gravity feed G_2 is passing through the electrode. The portion AB represents the sudden increase in current as a result of the oxidation of the iodide ions present in the reaction mixture. The maximum current represented by the point B corresponds to the concentration of iodide ions at the instant of opening the stopcock S_1, as already discussed. The portion BCE of the curve represents the decrease in the concentration of the iodide ions with the progress of the reaction.

It should be kept in mind that the maximum current represented by the point B corresponds to time t_1 and not to time t_2. The time interval $t_2 - t_1$ corresponds to the time period between the opening of the stopcock S_1 and the recording of the steady value of the limiting current for that iodide concentration on the recorder chart. This time period must be eliminated when working out the kinetics of the reaction.

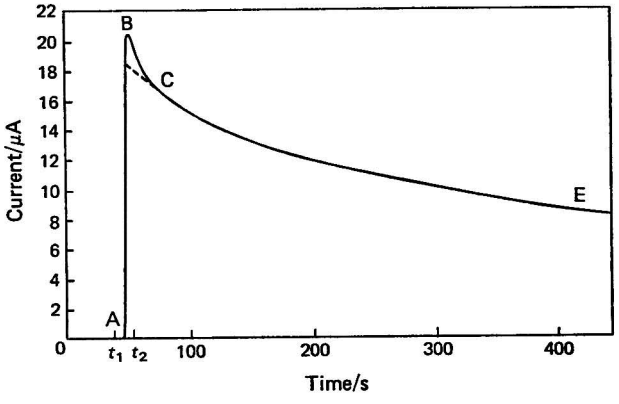

Fig. 2. Automatically recorded current - time graph for the oxidation of 0·02 M potassium iodide (100 ml) with 0·02 M hydrogen peroxide (100 ml) in acidic medium.

It should also be noted that the gravity feed G_1 provides a constant-pressure head only when the air, after displacing the liquid contained in the tube T_1, starts to bubble out of its lower end. Before that stage the pressure head is not constant. This accounts for the somewhat irregular portion BC of the curve BCE. It is thus only the portion CE of the graph that represents values of limiting currents obtained at a constant flow-rate of the solution. Hence it is this portion which should be taken into account when studying the kinetics of the reaction.

The extent of the irregular portion of the curve can easily be minimised by decreasing the diameter of the glass tube T_1. The smaller the diameter, the less is the amount of solution contained in it and hence the time required for displacement of that solution.

The bubbling of air into the solutions serves an additional but important purpose. It keeps the solution in the reaction vessel properly stirred. By decreasing the diameter of the tube, the rate of bubbling increases and this provides for better stirring. Thus, the gravity-feed device not only provides a constant-pressure head and thereby a constant flow-rate of the solution but it also provides for automatic stirring of the reaction mixture, which is essential in the study of kinetics.

Comparison of the Kinetic Data Obtained Voltammetrically with Those Obtained Titrimetrically

It was thought of interest to compare the kinetic data obtained voltammetrically for the reaction under investigation with those obtained titrimetrically. For this purpose, the current - time data of the graph CE were converted into concentration - time data, making use of the relationship between current and concentration obtained in the blank experiment and also of the Levich equation for the tubular electrode,[1,6,7] so that differences in the working parameters (*e.g.*, flow-rate and electrode length) in the two experiments would be taken into account. The concentration - time data thus obtained are recorded as a graph in Fig. 3.

The kinetics of the selected reaction were also studied by following the usual titrimetric procedure. The concentration - time data obtained in this way are also shown in Fig. 3. It is interesting to note that the concentration - time graphs obtained are almost superimposable. As the initial concentrations of both of the reactants were the same in both experi-

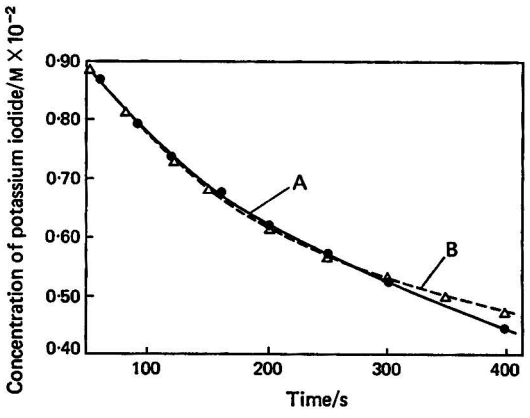

Fig. 3. Comparison of the kinetic data for the oxidation of potassium iodide with hydrogen peroxide obtained voltammetrically (curve A) by use of automatic methods with those obtained titrimetrically (curve B) in the usual way.

ments, the rate of reaction would be the same in both instances. Therefore, the concentration of potassium iodide at different intervals of time, whether determined voltammetrically or titrimetrically, should be the same provided that the mixing and dilution of the reaction mixture in the mixer is perfect. The almost complete superimposition of the two concentration - time graphs clearly shows that this is so. These observations confirm the dependability of the technique that has been developed for the automatic recording of the current - time graph (which is equivalent to the concentration - time graph) in the study of kinetics of reactions involving electro-active species.

TABLE I

KINETICS OF THE OXIDATION OF POTASSIUM IODIDE WITH HYDROGEN PEROXIDE IN 0·1 M SULPHURIC ACID

Volumes of KI and H_2O_2, 100 ml; potential applied to the electrode, 0·75 V *versus* S.C.E.; automatic dilution of the reaction mixture with the supporting electrolyte, 100 times; flow-rate of the diluted reaction mixture solution through the tubular electrode, 15 ml min^{-1}.

Time (t)/s	Limiting current/μA	Concentration of KI solution passing through the electrode/ M × 10^{-4}	Concentration of KI in reservoir G_1 at time t/M × 10^{-2}	Rate constant for second-order reaction/ 1 mol^{-1} s^{-1}
With initial concentrations of KI and H_2O_2 of 0·02 M—				
60	16·6	0·874	0·874	0·240
90	15·1	0·795	0·795	0·287
120	14·1	0·742	0·742	0·290
160	12·8	0·674	0·674	0·302
200	11·9	0·626	0·626	0·299
250	10·9	0·574	0·574	0·297
300	10·0	0·526	0·526	0·300
400	8·5	0·447	0·447	0·309
500	7·3	0·384	0·384	0·321
With initial concentrations of KI and H_2O_2 of 0·01 M—				
60	8·8	0·463	0·463	0·266
90	8·4	0·442	0·442	0·292
120	8·0	0·421	0·421	0·313
150	7·7	0·405	0·405	0·313
200	7·3	0·384	0·384	0·302
300	6·5	0·342	0·342	0·308
400	5·8	0·305	0·305	0·320

Analysis of the Current - Time Graph

The portion CE of the current - time graph (Fig. 2) was analysed in order to study the kinetics of the reaction under investigation. The relevant data are given in Table I. The oxidation of potassium iodide with hydrogen peroxide in an acidic medium is known to be a second-order reaction.[8] This order is confirmed by the constancy of the values for the second-order rate constants obtained in the present investigations and given in the last column of Table I. The average value of the rate constant is 0·30 l mol^{-1} s^{-1}.

The oxidation of potassium iodide was also studied for initial concentrations of potassium iodide and hydrogen peroxide of 0·01 M (with 0·1 M sulphuric acid). The voltammetric current - time graph was recorded automatically, as before, and then analysed. The data obtained are given in Table I. It can be seen that the values of the rate constants corresponding to different time intervals are close to the average value of 0·30 l mol^{-1} s^{-1}.

For a further standardisation of the automatic technique, the current - time graphs were obtained for different initial concentrations of potassium iodide and hydrogen peroxide. On analysis of each of the curves the order of the reaction was found to be 2 and the average value for the rate constant to be 0·30 l mol^{-1} s^{-1}.

Table II

KINETICS OF THE OXIDATION OF POTASSIUM HEXACYANOFERRATE(II) WITH HYDROGEN PEROXIDE IN 0·1 M SULPHURIC ACID

Volume of each solution, 100 ml; potential applied to the electrode, 0·4 V *versus* S.C.E.; automatic dilution of the reaction mixture with the supporting electrolyte, 100 times; flow-rate of the diluted reaction mixture solution through the tubular electrode, 15 ml min^{-1}; initial concentration of hexacyanoferrate(II) solution, 0·02 M; initial concentration of H_2O_2 solution, 0·02 M.

Time (t)/s	Limiting current/μA	Concentration of hexacyanoferrate(II) solution passing through the electrode/ M × 10^{-4}	Concentration of hexacyanoferrate(II) in reservoir G_1 at time t/M × 10^{-2}	Rate constant for second-order reaction/ l mol^{-1} s^{-1}
60	12·2	0·813	0·813	0·383
90	10·8	0·720	0·720	0·432
120	9·9	0·660	0·660	0·429
150	9·1	0·607	0·607	0·432
200	8·0	0·533	0·533	0·438
250	7·2	0·480	0·480	0·433
300	6·5	0·433	0·433	0·437

Table III

KINETICS OF THE OXIDATION OF SODIUM FORMATE WITH POTASSIUM PERMANGANATE IN 0·1 M SULPHURIC ACID

Volume of each solution, 100 ml; potential applied to the electrode, 0·40 V *versus* S.C.E.; automatic dilution of reaction mixture with the supporting electrolyte, 100 times; flow-rate of the diluted reaction mixture through the tubular electrode, 15 ml min^{-1}; concentration of sodium formate solution, 0·2 M; concentration of potassium permanganate solution, 0·004 M.

Time (t)/s	Limiting current/μA	Concentration of $KMnO_4$ solution passing through the electrode/ M × 10^{-5}	Concentration of $KMnO_4$ in reservoir G_1 at time t/M × 10^{-3}	Concentration of sodium formate in reservoir G_1 at time t/M × 10^{-3}	Rate constant for second-order reaction/ l mol^{-1} s^{-1}
60	13·4	1·489	1·489	98·7	0·0208
90	12·2	1·356	1·356	98·04	0·0180
150	10·3	1·144	1·144	97·85	0·0160
200	8·9	0·989	0·989	97·48	0·0150
300	6·5	0·720	0·720	96·80	0·0146
400	4·5	0·500	0·500	96·25	0·0149
500	3·2	0·356	0·356	95·87	0·0151
600	2·2	0·244	0·244	95·6	0·0153

The automatic technique described above was also tried in studying the kinetics of the oxidation of potassium hexacyanoferrate(II) with hydrogen peroxide and of the oxidation of sodium formate with potassium permanganate, both reactions taking place in 0·1 M sulphuric acid. The relevant portions of the automatically recorded current - time graphs were analysed as before and the corresponding data are recorded in Tables II and III. In both instances the results obtained are encouraging. It was found that both reactions were of second order with a rate constant of 0·43 l mol^{-1} s^{-1} for the oxidation of the hexacyanoferrate(II) and of 0·015 l mol^{-1} s^{-1} for the oxidation of sodium formate.

References

1. Sharma, L. R., and Jatinder Dutt, *Indian J. Chem.*, 1968, **6**, 593.
2. Sharma, L. R., and Jatinder Dutt, *Indian J. Chem.*, 1968, **6**, 597.
3. Sharma, L. R., and Jatinder Dutt, *Indian J. Chem.*, 1969, **7**, 485.
4. Sharma, L. R., and Jatinder Dutt, *Indian J. Chem.*, 1970, **8**, 173.
5. Sharma, L. R., Jatinder Dutt, and Inderjit Nirdosh, *Br. Chem. Engng.*, 1969, **14**, 1220.
6. Levich, V. G., "Physico-Chemical Hydrodynamics," Prentice Hall, Englewood Cliffs, New Jersey, 1962.
7. Blaedel, W. J., Olson, C. L., and Sharma, L. R., *Analyt. Chem.*, 1963, **35**, 2100.
8. Glasstone, S., "Textbook of Physical Chemistry," Second Edition, Macmillan & Co., London, 1974.

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Analytical Methods Committee

REPORT PREPARED BY THE METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

The Use of 50 per cent. Hydrogen Peroxide for the Destruction of Organic Matter (Second Report)

The Analytical Methods Committee has received and approved for publication the following Report from its Metallic Impurities in Organic Matter Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Dr. L. E. Coles (Chairman), Mr. W. Cassidy, Mr. P. M. Coleman, Mr. G. Collett, Dr. W. H. Evans, Mr. S. Greenfield, Mr. W. H. Hill, Mr. D. A. Lambie, Dr. R. F. Milton, Mr. B. E. Pearce, Mr. W. L. Sheppard and Mr. C. A. Watson, with Mr. P. W. Shallis as Secretary and Mr. J. J. Wilson as Assistant Secretary.

Introduction

In 1967 the Sub-Committee published its first Report[1] on the use of 50 per cent. hydrogen peroxide for the destruction of organic matter. With the increase in demand for methods of analysis involving wet digestion as a preliminary stage, it has been considered desirable to update the original report, drawing on the collective experience of the members of the Sub-Committee.

The first Report claimed certain advantages for the use of 50 per cent. hydrogen peroxide over the use of the more conventional oxidising acids, although it was realised that the use of this material was not without some hazard. The hazards involved and the safety precautions to be observed were detailed in the Report, and are not reproduced here in full. The Report gave specific procedures for the use of 50 per cent. hydrogen peroxide for the wet oxidation of A, plastics; B, readily oxidisable materials; C, soft drinks, etc.; D, syrups; and E, herbs, spices, gums, etc.

It must be emphasised that 50 per cent. (approximately 160-volume) hydrogen peroxide is a potentially dangerous chemical (see section on Hazards and Safety Precautions in the first Report[1]). Peroxide that has been splashed on to the skin can be "neutralised" with dilute ammonia solution, followed by a thorough rinse with water. This procedure should also be followed if there is any spillage of the hydrogen peroxide. It is a statutory requirement in the UK that eye protection must be worn by all workers using materials such as 50 per cent. hydrogen peroxide.[2] The advantages of the use of 50 per cent. hydrogen peroxide have been confirmed in practice, and the damage to fume cupboards has been found to be noticeably less than when the more conventional oxidising acids are used.

Most of the work done by the Sub-Committee in recent years has been on the application of atomic-absorption spectroscopy to the determination of metallic impurities in organic matter, and methods of wet oxidation have had to be adapted in order to analyse different types of organic material. Difficulties have been encountered in the analysis of fats and oils, foods with a high fat content, syrups and fruit concentrates. This Report deals with methods of wet digestion that have been developed by members of the Sub-Committee for the analysis of these types of materials. It must be stressed that in no circumstances should workers attempt to apply either of the procedures detailed in this Report to the wet oxidation of materials of types other than those specified. It is also extremely important that the amounts of sample and reagents specified and the details of the procedures given should be adhered to strictly.

Fats and Oils

The direct presentation to the flame, in atomic-absorption spectroscopy, of fats dissolved in organic solvents is useful only when the sensitivity for and the concentration of the metal under examination come within the ranges that can be measured by use of the instrument. Flameless techniques for fats and oils can give improved sensitivity, but often with less accuracy and precision. Background absorption problems occur with both techniques, particularly at wavelengths of less than 250 nm. It is therefore difficult to determine accurately the trace-metal content of, for instance, margarine that contains salt, or milk products containing large amounts of calcium and phosphorus. The analyst therefore requires a method for destroying the fats before extracting the trace metals with reagents such as 1,5-diphenylthiocarbazone (dithizone) or ammonium pyrrolidine dithiocarbamate.

Dry ashing as normally carried out on fats and oils results in ignition of the molten degraded material with consequent loss of trace metals. Ignition can be avoided, however, by pre-charring the samples in test-tubes held vertically on a hot-plate at 350 °C, followed by a gradual increase in temperature up to 550 °C in a closed (free from air turbulence) muffle furnace. Use of this procedure is restricted to samples of about 0·5 g, which does not improve the effective sensitivity of the analysis.

Wet-digestion techniques allow a larger mass of sample to be handled, but reactions can be violent. It must be emphasised that the use of perchloric acid for the wet digestion of fats and oils is particularly dangerous, and if nitric acid is used for the wet digestion there is the possibility that it will react with any glycerol liberated from the comparatively large sample of fat to form nitroglycerine. The usual practice of digestion in a Kjeldahl flask is wasteful of nitric acid, the bulk of which is expelled into the fume cupboard with deleterious effects on both the cupboard and the operator unless stringent precautions are taken. In addition, large volumes of acids are undesirable in determinations of trace metals as they introduce high blank values and tend to leach trace metals from the glassware.

The major problems normally encountered in wet digestions occur when too large a sample is contained in the reaction vessel. The method described here for fats and oils will effect a rapid and efficient wet oxidation based on the addition of small amounts of the sample followed by small amounts of peroxide to heated sulphuric acid.

Discussion

The method is based on the fact that molten fats and oils will form a thin layer on the surface of hot sulphuric acid. Many conical flasks have slightly domed bases, but it is advisable to use the minimum amount of sulphuric acid and therefore it is advantageous to select for the reaction a flask with the flattest possible base rather than a Kjeldahl flask, in order to extend the layer of acid. A degree of heating control is necessary, and this is best achieved by heating the flask on a hot-plate consisting of a sheet of steel plate mounted on a tripod over a Bunsen burner, as this allows the flask to be moved as necessary from a hot region to a cooler one.

As stressed in the first Report, peroxide must always be used in the presence of sufficient sulphuric acid to prevent violent oxidation. The first indication that too little sulphuric acid is present is that a dry froth advances up the sides of the flask, then small light flashes occur, followed by a rapidly accelerating reaction that expels vapours upwards. Safe conditions for the reaction are an excess of sulphuric acid, a limited amount of sample and just sufficient hydrogen peroxide to oxidise the sample; the use of a wide-necked flask is an additional safety measure. The best conditions for rapid and efficient oxidation are those in which the acid is maintained at gentle fuming during the digestion of each small increment of sample, but care must be taken to avoid heating the acid too strongly as oxidation by peroxide is less efficient at higher temperatures and becomes relatively ineffective if charring occurs. Oxidation of a charred mass can, however, be assisted by adding a few drops of concentrated nitric acid and carefully heating until all nitrous fumes have been expelled. No problems have been experienced in the digestion of 3–4 g of margarine with about 25 ml of hydrogen peroxide by the method described below. It is, however, desirable and sometimes essential that all residual hydrogen peroxide is removed by the addition of a suitable reducing agent such as sulphur dioxide.

Method A

Caution. Strict adherence to the conditions of the procedure is essential, otherwise the method is potentially hazardous.

Apparatus

Conical flasks. Wide-necked, volume 500 ml.
Hot-plate. A plate of 5-mm sheet steel (20 × 20 cm), mounted on a tripod over a gas burner is particularly suitable.
Pasteur pipettes.
Glass rods.
Measuring cylinder.

Reagents

Sulphuric acid, concentrated. Any grade of known low metals content can be used.
Hydrogen peroxide, 50 per cent. m/V. Store in a refrigerator.

Procedure

From a measuring cylinder transfer 5 ml of sulphuric acid to the conical flask, place the flask on the hot-plate, and apply heat. Adjust the position of the flask on the hot-plate so that the acid just evolves fumes. For an oil sample place a Pasteur pipette in the sample container or for a fat sample place two glass rods in the sample container, and weigh the container and contents.

Transfer about 0·10 g of the sample on the surface of the acid in the flask, and swirl the flask until a film is formed on the surface of the acid. Partially fill a long Pasteur pipette with hydrogen peroxide, insert the pipette into the flask so that its tip is about 3 cm above the surface of the acid, and add hydrogen peroxide dropwise to different areas of the surface until the acid is colourless. Continue heating until all bubbling ceases and until acid fumes are evolved. Add approximately another 0·10 g of sample and digest in the same manner. (If, during the reaction, there is any evidence of insufficient sulphuric acid being present in the flask, add more acid before making further additions of either sample or peroxide.)

Continue digesting small portions of the sample until sufficient has been oxidised for the analysis. Re-weigh the sample container and contents. Allow the digest to cool, and carefully dilute it with water.

Foods, Syrups and Fruit Concentrates

One of the samples used in a recent collaborative test carried out by the Sub-Committee was a cheese savoury baby food, which was specially chosen for its behaviour during wet oxidation as it is more difficult to oxidise than most foods. Similarly, problems arise in the digestion of syrups and fruit concentrates with high solids contents. If these samples are treated by the procedures previously recommended,[1] and especially if several samples are dealt with at the same time, there is a high risk of excessive charring with consequent wastage of time and reagents. The method recommended in this Report for fats and oils could be used for these materials, but the addition of essentially aqueous samples is more difficult to effect than the addition of fats and oils, which float on the surface of the acid.

The method described below involves the same basic principle of small and frequent additions, but in this instance the sample and peroxide are added together.

Discussion

The method depends on the miscibility of the sample with peroxide, which enables the reactants to be brought together in the reaction vessel simultaneously.

Concern has been expressed about the mixing of peroxide with sample and the possible hazard of ignition or explosion. It is a fact that contact between 50 per cent. hydrogen peroxide and anhydrous organic fibres or powders is liable to result in spontaneous combustion, and examples have been quoted of fires starting on paper tissues, cloths, mops and brooms that are used in dealing with spillage of the material. The samples for which this procedure is recommended, however, have a high proportion of water and, in the experience of one member of the Sub-Committee who has used this method for the last 7 years on about

5000 samples each year, there is no danger provided that the temperature of the mixture is not allowed to rise above ambient.

The safe conditions for the reaction are the same as for Method A, and the risk of violent oxidation is again minimised by making only small additions of the mixture at a time to the acid in the reaction vessel.

Method B

Caution. Strict adherence to the conditions of the procedure is essential, otherwise the method is potentially hazardous.

Apparatus

Beakers. Borosilicate glass, volume 100 ml.
Conical flasks. Borosilicate glass, volume 250 ml.
Pasteur pipettes.
Measuring cylinder.
Hot-plate.

Reagents

As for Method A.

Procedure

Place the weighed or measured sample into a beaker (usually 20 g or 20 ml will be sufficient, but a larger sample will improve the effective sensitivity of the method). Add an equal volume of hydrogen peroxide to the contents of the beaker, and mix well. **Do not heat this mixture or leave it unattended.**

From a measuring cylinder transfer 5 ml of sulphuric acid to a conical flask, and place the flask on the floor of a fume cupboard. Partially fill a Pasteur pipette with the sample mixture and add about 2 ml of it to the acid in the flask. The heat evolved on addition of the sample mixture will usually initiate the oxidation reaction; if it does not, place the flask on the hot-plate and warm gently until the reaction begins, and then return the flask to the floor of the fume cupboard. Continue to add portions of about 2 ml of the sample mixture to the acid at regular intervals so as to maintain the reaction.

After about five or six additions the volume of the reactants in the flask may have increased significantly. If this happens, transfer the flask to the hot-plate and complete the digestion with dropwise addition of peroxide alone until no further discoloration occurs. Return the flask to the fume cupboard floor, allow to cool, and then resume additions of the sample mixture. (If, during the reaction, there is any evidence of insufficient sulphuric acid being present in the flask, add up to a further 5 ml of acid before continuing with additions of the sample mixture.)

When all of the sample mixture has been added, place the flask on the hot-plate and complete the digestion with dropwise addition of peroxide until the fuming sulphuric acid is colourless. Allow the digest to cool, and carefully dilute it with water.

Conclusions

Since the publication of the first Report the experience gained by members of the Sub-Committee in the use of 50 per cent. hydrogen peroxide has been of great value in planning the collaborative experiments carried out. The revised procedures recommended in this report provide quick, efficient and clean methods of preparing difficult samples for analysis, the skills required being easy to acquire. The methods can be applied with confidence by suitably instructed laboratory staff.

Pressure-digestion Vessels

The use of pressure-digestion vessels for the wet oxidation of organic matter with 50 per cent. hydrogen peroxide cannot be recommended. These vessels are PTFE-lined steel bombs that can be securely closed either by screw-threaded barrels or with high-tensile steel bolts. They are designed primarily for dissolving ores and metals in oxidising acids. An experiment designed to check the efficiency of oxidation with hydrogen peroxide in such a container resulted in violent fracture of the eight securing bolts. A sample of milk (4 ml) was placed

in the vessel and dried overnight at 98 °C; 10 ml of sulphuric acid were added, the vessel and contents were cooled to −10 °C, and 20 ml of 50 per cent. hydrogen peroxide, also at −10 °C, were added. The bomb was sealed and shaken at intervals while the temperature rose to ambient; when opened, it was found that no digestion had occurred. The bomb was re-sealed and placed in an oven at 60 °C for 2 h; when opened after cooling it was found that digestion had not reached completion. A further 10 ml of peroxide were added, the bomb was re-sealed, placed in a muffle furnace and heated only gradually from ambient. At about 80 °C an explosion occurred. The eight securing bolts had fractured and the lid of the vessel had been projected into the lining of the furnace.

References

1. Analytical Methods Committee, *Analyst*, 1967, **92**, 403.
2. "The Protection of Eyes Regulations 1974," S.I. 1974 No. 1681, H.M. Stationery Office, London.

Communication

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication precludes the use of diagrams, but tables and formulae can be included. Communications should not be simple claims for priority: this facility for rapid publication is intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.

Manuscripts are not subjected to the usual examination by referees and inclusion of a Communication is at the Editor's discretion.

Changes in Absorbance Values of Solutions of Brilliant Green After Initial Dissolution of the Solid: Precaution to be Taken in its Use as a Colorimetric Reagent

Brilliant Green is used extensively as a reagent for the extraction of anions,[1–3] and certain precautions to be taken in its use owing to its acid - base properties and to the uncertain purity of commercial samples have been suggested.[4] During work in these laboratories, it was observed that the absorbances at 625 nm of certain aqueous solutions of Brilliant Green increased about five-fold over a period of about 1 h after preparation. This effect would not normally cause incorrect results to be obtained when Brilliant Green is used as an extractive photometric reagent provided that a sufficient excess were taken and, for this reason, the effect, which is caused by the slow de-dimerisation of Brilliant Green, has not been reported previously.

Recently, however, we were approached about the use of the apparent molar absorptivity of Brilliant Green samples as a criterion for selecting suitable commercial samples for use as a reagent. For this reason, and also because there is some risk of using an insufficient volume of reagent solution in analytical procedures, it was felt that this communication was justified. The presence of a considerable amount of dimer in the aqueous solution effectively reduces the amount of Brilliant Green available for extraction, and in instances when the analyst uses only a small apparent excess of Brilliant Green, there may be a deficiency of the monomer in solution.

Experimental and Results

Absorbance measurements were made with a Unicam SP600 spectrophotometer, and glass cells of 1 cm path length were used (except where indicated otherwise). A sample of BP-grade Brilliant Green was used. Absorbance values of several aqueous and ethanolic solutions of Brilliant Green are given in Table I, from which several facts are apparent. Firstly, when a 10^{-5} M aqueous solution is prepared quickly from the solid, the full absorbance at 625 nm of the Brilliant Green monomer is obtained. If, however, the 10^{-5} M solution is prepared from an "aged" 10^{-3} M aqueous stock solution, a considerably lower absorbance is obtained initially. The absorbance of this 10^{-5} M solution increases with time and approaches its full value after 18 h; the increase in absorbance during the first hour is shown in Table II.

TABLE I

APPARENT MOLAR ABSORPTIVITIES OF BRILLIANT GREEN SOLUTIONS MEASURED AT A TOTAL BRILLIANT GREEN CONCENTRATION OF 10^{-5} M AT 625 nm

Length of time at 10^{-3} M before dilution to 10^{-5} M/h	Subsequent length of time at 10^{-5} M before making measurement*/h	Apparent molar absorptivity/10^5 l mol^{-1} cm^{-1}	
		Aqueous solutions	Ethanolic solutions
0	0	1·005	0·962
18	0	0·286	0·964
	18	0·960	0·152
42	0	0·237	1·002
	24	0·842	0·106
	42	0·982	0·076

* Stored in the dark.

The effect of an increased "ageing time" of the 10^{-3} M solution in reducing still further the initial absorbance of the 10^{-5} M solution is shown in Table I, and it is also apparent that the 10^{-5} M solution does not deteriorate when stored in the dark for 2 d. Full absorbances are observed for 10^{-5} M ethanolic solutions when they are prepared rapidly from the solid or from an "aged" 10^{-3} M solution in ethanol. The 10^{-5} M ethanolic solution deteriorated rapidly even when it was stored in the dark.

TABLE II

EFFECT OF DE-DIMERISATION OF BRILLIANT GREEN ON THE APPARENT MOLAR ABSORPTIVITY OF A 10^{-5} M AQUEOUS SOLUTION PREPARED BY DILUTION FROM AN "AGED" 10^{-3} M SOLUTION

Time after dilution from 10^{-3} M/min	1	5	10	15	25	40	50	60
Apparent molar absorptivity at 625 nm/10^4 l mol^{-1} cm^{-1}	2·02	2·85	3·58	4·44	5·58	6·82	7·28	7·70

It is clear that the rates of dimerisation and de-dimerisation of Brilliant Green in aqueous solution are slow. In an equilibrated 10^{-3} M aqueous solution, a considerable proportion (about 80 per cent.) of the Brilliant Green is dimerised. At 10^{-5} M concentration, however, although the equilibrium concentration of the dimer is very small, the dimer formed in the more concentrated solutions requires at least 1 h to dissociate into the monomer.

The immediate production of the full absorbance when concentrated solutions of Brilliant Green in ethanol are diluted with ethanol indicates either that dimerisation and de-dimerisation in ethanol are rapid, or that dimers are not formed in this solvent. The fact that the full absorbance is obtained immediately when the 10^{-3} M ethanolic stock solution is diluted to 10^{-5} M with water supports the latter explanation. Rabinowitch and Epstein[5] showed that Methylene Blue forms dimers in aqueous but not in ethanolic solutions.

Poluektov *et al.*[6] determined the dimerisation constants of several basic dyes, including Brilliant Green, and gave the $\lambda_{max.}$ values for the latter as 627 nm (monomer) and 590 nm (dimer), but did not give molar absorptivities for the two species. From measurements of absorbance at 625 and 585 nm in the present work (see Table III), the molar absorptivity of the dimer at 585 nm is estimated to be about two orders of magnitude lower than that of the monomer at 625 nm. Thus, $\epsilon_{apparent}/\epsilon_{max.}$ at 625 nm, where $\epsilon_{max.} = 10^5$ l mol^{-1} cm^{-1}, is a good measure of the proportion of monomer present in the solution. From this assumption, it follows that the concentration of dimer in a solution is proportional to $A_{max.} - A$. When log $(A_{max.} - A)$ is plotted against time using the results in Table II, a straight line is obtained, indicating that the de-dimerisation reaction obeys first-order kinetics.

TABLE III

EFFECT OF CONCENTRATION ON APPARENT MOLAR ABSORPTIVITIES AT 585 AND 625 nm

Path length of cell/cm	Total Brilliant Green concentration in measured solution/M	Apparent molar absorptivity of equilibrated solutions/10^5 l mol^{-1} cm^{-1}		Ratio, $A_{585\ nm}:A_{625\ nm}$
		585 nm	625 nm	
4	2×10^{-6}	0·34	0·72	0·42
1	10^{-5}	0·33*	0·77*	0·46
0·1	8×10^{-5}	0·29	0·58	0·51

* Corresponding values immediately on dilution were 0·13 and 0·27, respectively ($A_{585\ nm}:A_{625\ nm} = 0·47$).

The effect of using only a small apparent excess of Brilliant Green in the determination of 50 μg of perrhenate by means of a previously published method[4] was studied. A 0·025 per cent. solution of Brilliant Green was used as reagent. The total amounts of Brilliant Green and perrhenate used were $0·52 \times 10^{-6}$ and $0·2 \times 10^{-6}$ mol, respectively. When an "aged" 0·025 per cent. ethanolic solution was used, the absorbance obtained was equivalent to the full molar absorptivity of the Brilliant Green, 10^5 l mol^{-1} cm^{-1}, whereas with a 0·025 per cent. aqueous solution, the absorbance was equivalent to an apparent molar absorptivity of only $0·7 \times 10^5$ l mol^{-1} cm^{-1}. It is apparent that only the monomeric form of Brilliant Green is extractable.

The following basic dyes were examined for signs of slow dimerisation and de-dimerisation: Rhodamine B, Butyl Rhodamine B, Rhodamine 6G, Rhodamine 3G, Sevron Brilliant Red, Sevron Brilliant Red 4G, Sevron Bordeaux G, Methylene Blue, Dimethylmethylene Blue and Crystal Violet. Most of these dyes are known to form dimers at higher concentrations,[6] but none of them exhibited a slow de-dimerisation effect similar to that of Brilliant Green. Solutions prepared by dilution of "aged" concentrated solutions gave absorbances similar to those prepared directly from the solid.

Discussion

Proton addition to Brilliant Green had been shown previously to be a relatively slow reaction.[4] From the work described here, it is apparent that the dimerisation and de-dimerisation reactions are also slow, so that care must be taken in determining apparent molar absorptivities of samples of Brilliant Green in aqueous solution. The dilute solution of which the absorbance is measured must be prepared rapidly from the solid, or time must be allowed for the de-dimerisation to be completed. Ideally, the dilute solution should be prepared as rapidly as possible from the solid, and any increase in absorbance within 1 h should be noted. Owing to the susceptibility to decomposition of dilute ethanolic solutions of Brilliant Green, measurements in this solvent are not recommended.

Because of the formation of the inert dimer in concentrated aqueous stock solutions of Brilliant Green, the use of freshly prepared concentrated ethanolic stock solutions is to be preferred. This practice is not always feasible, however, owing to the increased blanks obtained with some extraction solvents in the presence of ethanol. When an aqueous stock solution must be used, it should be remembered that only about one fifth of the total Brilliant Green present is available for extraction at the 10^{-3} M level.

References

1. Fogg, A. G., Burgess, C., and Burns, D. T., *Talanta*, 1971, **18**, 1175.
2. Blyum, I. A., and Pavlova, N. N., *Zav. Lab.*, 1963, **29**, 1407.
3. Blyum, I. A., and Oparina, L. I., *Zav. Lab.*, 1970, **36**, 897.
4. Fogg, A. G., Burgess, C., and Burns, D. T., *Analyst*, 1970, **95**, 1012.
5. Rabinowitch, E., and Epstein, L. F., *J. Am. Chem. Soc.*, 1941, **63**, 69.
6. Poluektov, N. S., Bel'tyukova, S. V., and Meshkova, S. B., *Zh. Analit. Khim.*, 1971, **26**, 1042.

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Book Reviews

ELECTRON MICROPROBE ANALYSIS. By S. J. B. REED. *Cambridge Monographs on Physics.* Pp. xvi + 400. Cambridge, London, New York and Melbourne: Cambridge University Press. 1975. Price £12.

For an excessively long period, electron microprobe spectroscopists have been without an authoritative guide to their techniques and instruments. This has resulted in the formation of highly commendable and vigorous user groups and a very effective "underground" communication system between operators. On the other hand, this system has tended to set them apart from other analysts, including even X-ray spectroscopists and electron microscopists. This fragmented state of affairs should be brought to an end by the publication of this long-awaited book by a world authority in the field and thus, if the metaphor may be forgiven, microprobe users will no longer be working in a vacuum. After some reading they should realise that many of their principles, techniques and computations are essentially similar to those of allied spectroscopists—sometimes simpler, sometimes more complex.

The author has presented the full story of the technique in an ordered manner in a depth that strikes a reasonable compromise between detailed original papers, instrument manuals and the superficial; some readers will argue that there is too little mathematics and some that there is insufficient qualitative description, but to the reviewers the balance seems fair for a book of this size. Thus, (*a*) the general principles and essential features of the instrument are reviewed, (*b*) descriptions and discussions of problems concerned with electron beam generation, focusing, scanning and backscattering are given, (*c*) X-ray fluorescence, absorption, dispersion and counting are treated in some detail and (*d*) particular attention is paid to correction procedures, light element analysis, analysis of thin films and non-dispersive methods.

The author gives a straightforward account in a strictly uncritical fashion so that certain matters of past and present controversy and particular difficulty do find themselves somewhat comminuted through the book, but it would be unkind to say that he has skated round these topics: the diligent reader should be able to arm himself with sufficient information to draw his own conclusions and, indeed, join happily in the arguments.

Useful data are tabulated, but always near the relevant discussion; a compendium of tables inserted as an appendix would be more useful if the work is to be regarded as a source book. Strangely, for a physicist, the author persists in supplying the data to every available significant figure when it is known that they are subject to appreciable error: readers should be wary of this tendency and of the weight to be attributed to certain correction operations. Otherwise, the book is clearly presented and is pleasantly readable. References, presented in the curious convention of physicists, are abundant and include some from 1975.

Despite the price the book is excellent value for money and is essential instrument-side reading to all experienced as well as budding microprobe users. P. R. MONK and D. A. PANTONY

BUFFERS FOR pH AND METAL ION CONTROL. By D. D. PERRIN and BOYD DEMPSEY. Pp. viii + 176. London: Chapman and Hall. 1974. Price £3·50.

Few chemical and biochemical processes can be studied effectively without proper control of pH. This implies the use of buffer solutions, and often the problem is which of the many available systems is the best to use for a particular purpose. The authors of this book, the second in a series of manuals on physical chemistry and biochemistry, set out to provide a source of information and instructions for the choice of suitable buffer substances and preparation of working solutions. A brief introduction is followed by a short account of the theory of buffer action, then sections are given on applications of buffers, practical limitations in their use, buffers for part-aqueous and non-aqueous systems, and metal-ion buffers, the purification of substances used in buffers and the preparation of buffer solutions. The book concludes with appendices listing composition - pH tables, thermodynamic acid-dissociation constants and the correct form of the Henderson - Hasselbalch equation.

This is a book that will be welcomed by all involved in the study of chemical equilibria in aqueous and non-aqueous systems, where pH control is essential. The extension into pM control is timely, not only because of increased concern in biochemical studies for strict control of "free" metal-ion concentrations but also for the calibration of ion meters utilising metal-ion electrodes.

WILLIAM I. STEPHEN

DETERMINATION OF GASEOUS ELEMENTS IN METALS. Edited by L. M. MELNICK, L. L. LEWIS AND B. D. HOLT. *Chemical Analysis Series, Volume* 40. Pp. viii + 744. New York, London, Sydney and Toronto: John Wiley & Sons. 1974. Price £19·25.

The difficult task of summarising the vast and still rapidly expanding subject of the determination of gases in metals has been tackled in this book by a team of over twenty specialists under the guidance of Dr. Melnick and his co-editors. They have successfully produced a fair degree of uniformity in the presentation of the twenty specialist chapters into which the book is divided.

The book commences with a very useful chapter on the solubility, diffusion and thermochemistry of gaseous elements in metals, which acts as the theoretical background to the later chapters dealing with specific methods of determination. The second chapter deals with theoretical aspects of the ubiquitous vacuum fusion method, and this is followed by a chapter dealing with activation analysis. Next comes an account of vacuum and inert gas fusion that deals with practical aspects of these techniques and with commercially produced apparatus. After a chapter covering optical and mass-spectrometric methods there are specialist reviews on the determination of hydrogen, oxygen and nitrogen.

A series of chapters follows, dealing with about 30 of the metallic elements altogether and having lateral or vertical proximity in the periodic table as the linking factor within each chapter. Iron and steel and ferro-alloys also have separate chapters and the book concludes with a contribution on nuclear and ion-beam methods for surface and near-surface reactions.

As well as theoretical considerations, and descriptions of instruments, many chapters also include detailed working instructions for analytical procedures. Each chapter is followed by a list of references, quoted in order of mention; the book has over 2000 in all. However, there is no author index, so the repetition of quotations cannot be gauged and it is difficult to look up the publications by a specific worker.

The breadth of field of the book presents difficulties to the reviewer as well as to the authors, but the coverage of the determination of gases in iron and steel and ferro-alloys, and the associated theory, is excellent. Naturally, there are areas where one disagrees with the author, and there are problems in keeping a book of this nature up to date, as the editors recognise in their preface. With this general approval in mind, the discussion that follows is intended as comment rather than criticism.

There is an extensive subject index, but this is difficult to use in practice. For instance, there are no entries under "Steel," and the relevant entries appear under "Iron and steel." No doubt growing familiarity with the book would ease this problem. Each of the separate chapters starts with a list of contents, and these are often the quickest route to a desired topic. Cross-references between chapters are routed via the lettered and numbered chapter sub-divisions, rather than page numbers, and this also causes difficulty in rapid access until the system has been mastered.

As mentioned earlier, the text dealing with commercial instruments will soon date unless revised fairly frequently. For example, the Balzers EA1 analyser is described for its application to hydrogen determinations but not the later specialised Model EAH 202 or its most recent version, EAH 220. The book also describes at least two instruments that have proved unsatisfactory in service. One might also quibble over the absence of comment on errors likely to arise when liquids such as paraffin or glycerol are used instead of mercury as entraining liquid in gas collecting systems (p. 294).

When dealing with chemical methods, the authors still recommend the Nessler reaction for ammonia in the determination of nitrogen, rather than the indophenol blue reaction now favoured in Europe, because of its increased sensitivity and lower background colour. There are frequent references to the determination of aluminium nitride by the methyl acetate - bromine dissolution method followed by distillation from sodium hydroxide medium, as originally described by Beeghly. Recent work in the UK has shown that the procedure is not specific for aluminium nitride and can give misleading results if so interpreted. The use of ultrasonic agitation as an aid to sample dissolution is also unrecorded.

Although the editors say that they have tried to make the units used in the text consistent throughout, it is still possible to find examples of most of the alternatives which have been used in the past. For example, on pages 16–19, hydrogen content is variously quoted in ml/100 g, cc/100 g, p.p.m. by weight and weight %. Both oxygen and nitrogen contents are given in p.p.m. or % in adjacent sections of the text. A draft British Standard aims to use % wt for the latter and p.p.m. for hydrogens; presumably, there is as yet no equivalent American proposal. There are very few printers errors, only the misprinting of "Union" and "and" on p. 123 having been noticed. There

are many formulae, neatly printed, but there seems to be an error in the example worked from equation (11) on page 95. Many of the diagrams and photographs have been taken from original publications, with due acknowledgment; this leads to considerable but not obtrusive variation in style.

The book jacket claims to show how to select proper methods and instrumentation for determining gases in any of a large number of metals, and the book to be written for analysts and supervisors in departments performing such analyses. This seems a fair summary of the aim of the authors, and in the main the result is successful; it is hoped that it will reach the appropriate readership and that the authors will be able, from time to time, to make revisions, thus keeping it up to date.

J. D. HOBSON

PRACTICAL INORGANIC CHEMISTRY. PREPARATIONS, REACTIONS AND INSTRUMENTAL METHODS. By GEOFFREY PASS AND HAYDN SUTCLIFFE. Second Edition. Pp. xvi + 239. London: Chapman and Hall Ltd. 1974. Price £2·60.

Recent years have seen a new emphasis in the teaching of chemistry to include at least some "pure" inorganic practical work as opposed to the "applied" inorganic work that is traditionally part of courses in practical analytical chemistry. With the movement away from classical reaction chemistry towards more and more instrumentation in analytical processes, students easily forget (or have never known) the properties and reactions of the elements and their compounds. If a practical course in inorganic chemistry can help to redress this imbalance in basic chemical knowledge, then its inclusion in college and university chemical curricula is something to be desired. Such courses demand suitable text-books and this book, now in its second edition and up-dated to include SI units, appears to be an admirable student text for laboratory use. It describes preparations, reactions and instrumental methods that are representative of present-day philosophy. The 22 chapters cover such topics as the reactions of transition metals, preparations and examinations of co-ordination compounds, clathrate compounds (note that a 5-μl not 5-ml sample should be taken for gas - liquid chromatography of the quinol - sulphur dioxide clathrate), stabilisation of oxidation states, stereochemistry, non-aqueous chemistry, inorganic polymers and techniques of spectroscopy, magnetic susceptibility and electrochemical measurements.

Each experiment has some additional and complementary work that should encourage the student to extend both his interest and knowledge by reading of original literature and other relevant sources. In fact, this supplementation of information is essential if the student is to obtain the most benefit from this book. Its remarkably low price is a great encouragement to the ever impecunious student to purchase his own copy, and teachers interested in including some "pure" inorganic experiments in their practical chemistry courses will find in this book many interesting experiments to choose from.

WILLIAM I. STEPHEN

ACTUALITÉS DE CHIMIE ANALYTIQUE. ORGANIQUE, PHARMACEUTIQUE ET BROMATOLOGIQUE. 23e Série. Edited by J.-A. Gautier, P. Malangeau and F. Pellerin. Pp. iv + 241. Paris: Masson et Cie. 1975. Price 150F.

This French book is one of a continuing series of selected reviews in various fields of analytical chemistry. The present volume contains five miscellaneous contributions dealing with (1) polarographic studies of the reactions of some halogenoquinones (J. Bonastre and S. Tellier); (2) applications of specific electrodes in pharmaceutical, bromatological and medical analysis (J. Mertens *et al.*); (3) organic peroxides—recent analytical applications in the field of lipids and solvents (V. Karnojitsky); (4) use of vapour-phase chromatography in toxicology and in the analysis of drugs (J. Lebbe); and (5) recent advances in the liquid-phase chromatography of amino-acids and peptides (R. Munier).

Each review appears to be a fairly straightforward attempt to cover the relevant literature up to about 1972–73 primarily for French-language readers. The treatment is in no way exceptional and follows that of conventional English-language reviews of the subjects published previously. The review on specific electrodes, in fact, covers only the electrodes themselves and those readers interested in applications must wait for the next volume.

As a paperback, the book is not very well put together, the binding gum being very inferior for holding the cover and pages together. At the present rate of exchange (about 9F to £1), this is an extremely expensive volume in the UK and it is really only of interest to those French-speaking chemists who have no access to similar reviews in English. Its appeal to English-speaking chemists is minimal and it cannot, in its poorly presented state, be recommended for library use.

WILLIAM I. STEPHEN

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