The Analyst

The Analytical Journal
of The Chemical Society

A monthly international publication dealing
with all branches of analytical chemistry

Volume 105 No 1247 Pages 97–192 February 1980

THE ANALYST

THE ANALYTICAL JOURNAL OF THE CHEMICAL SOCIETY

Published by The Chemical Society

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

Advertisements: Advertisement Department, The Chemical Society, Burlington House, Piccadilly, London, W1V 0BN. Telephone 01-734 9864

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Volume 105 No 1247 February 1980

Summaries of Papers in this Issue

Examination of Petroleum Products of High Relative Molecular Mass for Forensic Purposes by Synchronous Fluorescence Spectroscopy. Part I. Appraisal of Experimental Factors

The luminescence of petroleum derivatives is discussed with reference to a standard technique for their differentiation based on synchronous fluorescence spectroscopy. The spectra are highly sensitive to quenching by oxygen and by halogenated compounds; for general-purpose use, solutions of samples (in hydrocarbon solvents) must be deoxygenated. Although the quantum yield of the fluorescence is of the order of 0.15, the fluorescence is distributed over a wide wavelength range and, at the sample concentrations usually required in order to obtain satisfactory spectra, significant inner filter effects are often present and corrections must be made for these. Various spectral features that differ between samples are defined. After correction for instrumental and inner filter effects, the features are expressed numerically for evaluation and use in the pattern recognition study that will be described elsewhere in Part II. Examples of other types of spectra are considered in relation to the standard technique. These are synchronous and conventional fluorescence contour diagrams, synchronous derivative spectra, low-temperature synchronous fluorescence spectra and phosphorescence spectra.

Keywords: *Fluorescence*; *phosphorescence*; *synchronous fluorescence spectroscopy*; *petroleum products*; *forensic analysis*

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Analyst, 1980, **105**, 97–190.

Determination of Tetramethyl- and Tetraethyllead Vapours in Air Following Collection on a Glass-fibre - Iodised Carbon Filter Disc

Methods are described for the determination of tetraalkyllead vapour in air. The related sampling procedure operates efficiently over a wide range of lead-in-air concentrations, flow-rates and sampling periods. It is also effective under extreme conditions of humidity, can be applied in the presence of gasoline vapour and is especially convenient for personal monitoring or for use in the field.

The air sample is passed through a glass-fibre - iodised carbon filter on which tetraalkyllead compounds are collected. Lead is then extracted from the filter and converted into the inorganic state by treatment with iodine solution, followed by colorimetric determination as lead dithizonate using a commercially available test kit. In an alternative procedure involving the use of nitric acid - bromine reagent for extraction, the lead is determined by atomic-absorption spectrophotometry with electrothermal atomisation.

The efficiency of the methods has been confirmed using laboratory prepared air samples containing 5–400 $\mu g\ m^{-3}$ of lead.

Keywords: *Tetramethyllead determination*; *tetraethyllead determination*; *air analysis*

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Analyst, 1980, **105**, 110–118.

Determination of Copper, Lead, Cadmium, Nickel and Cobalt in EDTA Extracts of Soil by Solvent Extraction and Graphite Furnace Atomic-absorption Spectrophotometry

A procedure is described for the determination of copper, lead, cadmium, nickel and cobalt in EDTA extracts of soil and similar material. Diethylammonium diethyldithiocarbamate or ammonium tetramethylene dithiocarbamate metal complexes are extracted into xylene from the EDTA extracts, and metals are determined in the xylene phase by atomic-absorption spectrophotometry using a graphite furnace atomiser. The detection limits (concentrations in soil) are approximately copper 0.8, lead 0.3, cadmium 0.07, nickel 2.5 and cobalt 0.8 $\mu g\ g^{-1}$. These detection limits might be improved by at least a factor of 10. Iron, manganese, aluminium, calcium and zinc do not interfere in amounts likely to be found in extracts of natural or contaminated soils.

Keywords: *Copper, lead, cadmium, cobalt and nickel determination*; *solvent extraction*; *atomic-absorption spectrophotometry*; *graphite furnace atomisation*; *soil analysis*

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Analyst, 1980, **105**, 119–124.

Simultaneous Acid Extraction of Six Trace Metals from Fish Tissue by Hot-block Digestion and Determination by Atomic-absorption Spectrometry

A simple and rapid digestion method is reported for the simultaneous acid extraction of chromium, copper, zinc, cadmium, nickel and lead from high-fat fish tissue. Samples are digested with nitric and sulphuric acids at 150 °C in a modified aluminium hot-block. The method is specially set up for fish sample sizes of up to 5 g, for low level detection of these elements. After digestion, acid extracts of the sample are analysed by direct flame atomic-absorption spectrometry for copper, zinc and chromium. The other three elements, cadmium, nickel and lead, are concentrated by chelation with ammonium tetramethylene dithiocarbamate followed by solvent extraction with isobutyl methyl ketone and determined by flame atomic-absorption spectrometry. The ease, rapidity and safety by which samples can be processed by this method make it suitable for the routine preparation of a large number of samples simultaneously.

Keywords: *Trace metal determination*; *fish analysis*; *hot-block digestion*; *atomic-absorption spectrometry*

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Analyst, 1980, **105**, 125–130.

Rapid Spectrophotometric Method for the Determination of Monofluorophosphate

A method is described for the rapid spectrophotometric determination of orthophosphate in the presence of monofluorophosphate. Subsequent acid hydrolysis of monofluorophosphate enables this ion to be determined in the same sample. The technique is based on a single-reagent modification of the molybdophosphovanadate procedure. Using this method as described it is possible to determine up to 0.5 mg of phosphorus but this can be extended to 1.8 mg of phosphorus using an alternative procedure. Free phosphorus can be determined in the presence of up to a 50-fold excess of monofluorophosphate. The method is especially well suited for following hydrolysis reactions of monofluorophosphate.

Keywords: *Monofluorophosphate determination*; *orthophosphate*; *spectrophotometry*; *molybdophosphovanadate reagent*

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Analyst, 1980, **104**, 131–138.

Automatic Determination of Boron (0.10–10.0 mg l^{-1}) in Raw and Waste Waters

An automated method, employing azomethine-H in aqueous medium, for the determination of boron in raw waters and effluents is described. The method is capable of a limit of detection of 0.1 mg l^{-1} and the response is essentially linear to 4.0 mg l^{-1}, although a calibration graph is required above this level. A wide range of possible interferences was tested, but none proved of practical importance except sample colour, for which adequate correction is provided. Recovery after spiking a typical range of raw and waste waters was adequate.

Keywords: *Boron determination*; *AutoAnalyzer*; *water analysis*

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Analyst, 1980, **105**, 139–146.

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FLAP A

FEBRUARY 1980 Vol. 105 No. 1247

The Analyst

Examination of Petroleum Products of High Relative Molecular Mass for Forensic Purposes by Synchronous Fluorescence Spectroscopy

Part I. Appraisal of Experimental Factors

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The luminescence of petroleum derivatives is discussed with reference to a standard technique for their differentiation based on synchronous fluorescence spectroscopy. The spectra are highly sensitive to quenching by oxygen and by halogenated compounds; for general-purpose use, solutions of samples (in hydrocarbon solvents) must be deoxygenated. Although the quantum yield of the fluorescence is of the order of 0.15, the fluorescence is distributed over a wide wavelength range and, at the sample concentrations usually required in order to obtain satisfactory spectra, significant inner filter effects are often present and corrections must be made for these. Various spectral features that differ between samples are defined. After correction for instrumental and inner filter effects, the features are expressed numerically for evaluation and use in the pattern recognition study that will be described elsewhere in Part II. Examples of other types of spectra are considered in relation to the standard technique. These are synchronous and conventional fluorescence contour diagrams, synchronous derivative spectra, low-temperature synchronous fluorescence spectra and phosphorescence spectra.

Keywords: *Fluorescence*; *phosphorescence*; *synchronous fluorescence spectroscopy*; *petroleum products*; *forensic analysis*

Since the work of Parker and Barnes[1] an appreciable number of uses of fluorescence spectroscopy has been made in the identification and determination of petroleum oils and related materials, mainly in the fields of oil pollution and forensic science. However, because the optical characteristics of the various types of spectrofluorimeter employed have varied greatly in the extent to which the spectra they produce are distorted (some examples are given in a recent paper by Eastwood *et al.*[2]), and because the fluorescence characteristics of the highly complex mixtures present in petroleums are still not well understood, the various published methods of analysis have produced results that are of necessarily restricted application.

Although the choice of analytical methods remains an essentially empirical undertaking, instrumentation that produces relatively undistorted spectra is now widely available commercially. Hence, a satisfactory degree of standardisation has become possible for the compilation of spectral data that are sufficiently reproducible and stable with time for their use in the classification of unknown samples and in the calculation of evidential significance, *e.g.*, by pattern recognition techniques. This will be considered in subsequent work. This paper reviews the experimental variables pertinent to these materials that must be defined and controlled before any attempt can be made to establish satisfactory collections of data.

Experimental

Instrumental Details

The spectra are recorded with a standard Perkin-Elmer MPF-4 spectrofluorimeter fitted with a corrected spectra accessory that, together with the various instrumental settings for wavelength and sensitivity, is calibrated essentially according to the manufacturer's manuals.

Both a standard lamp and a quantum counter have been used for the emission correction, without significant differences between the results.

Except where indicated otherwise, the spectra shown here are corrected on the excitation side and, therefore, represent the relative excitation efficiency *versus* wavelength in terms of photon units. The effect of the relatively smaller emission correction on an already excitation-corrected synchronous spectrum is indicated in Fig. 1(*c*).

The derivative spectra are obtained by electronic differentiation of the signal from the fluorimeter with an attachment made by Mr. D. A. Collins. A similar device was described by Green and O'Haver.[3]

Samples are presented to the fluorimeter, for perpendicular illumination, in a 5-mm square-sectioned Spectrosil cell (Starna Ltd., London), in which they can be deoxygenated by a stream of nitrogen passed into and out of the cell through 0.25 mm i.d. steel tubing sealed into an air-tight cover.[4] Provided that the inlet tubing, by means of which both sample solutions and nitrogen are introduced into the cell, is run down a junction of two cell faces, the spectra obtained should be independent of whichever face is set in the excitation beam.

Ultraviolet absorption spectra are recorded with a Pye Unicam SP8000 spectrometer, the wavelength and absorbance calibrations of which are periodically checked with, respectively, a holmium oxide filter and a potassium dichromate solution in aqueous sulphuric acid.[5]

A Cahn Gram Electrobalance is used in the preparation of solutions of known concentrations of oil. Samples to be weighed are transferred from a needle point to a 1 × 10 mm coil of 0.1-mm steel wire hung on one arm of the balance.

Glassware

Glassware with which samples and solvents come into contact is soaked in Decon 90, rinsed with distilled water and baked out overnight at a temperature of not less than 250 °C.

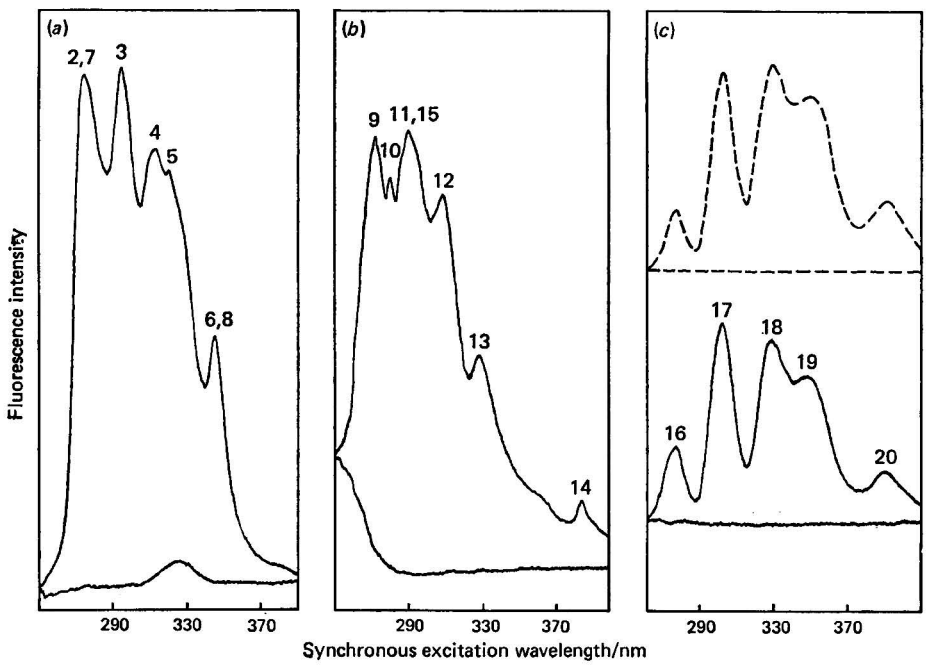

Fig. 1. Examples of synchronous fluorescence spectra obtained at scanning intervals of (*a*) 34, (*b*) 20 and (*c*) 7 nm from solutions of three different oils (20 μg ml^{-1}) in deoxygenated cyclohexane. The solvent base line is shown in each instance. The numbers on the spectra identify the featues defined in Table I. The full-line spectra are corrected for variation in the instrumental photon response on the excitation side. The broken-line spectra are both excitation- and emission-corrected.

Lower temperatures or shorter times are ineffective in the removal of trace amounts of fluorescent contaminants.

Materials

Nitrogen (BOC Ltd., "oxygen-free") is passed through a column packed with manganese(II) oxide on Kieselguhr[6] to remove trace amounts of oxygen that may have permeated the supply lines, and then through a pre-saturator containing the relevant solvent.

The hydrocarbon solvents are of commercial spectrophotometric grades. If, as is usual, extraneous fluorescence is present at an intensity greater than 20% of the Raman C–H signal synchronously excited at 34 nm,[7] the solvent is passed through a column of charcoal (Hopkin and Williams Ltd., "for gas absorption") activated at 400 °C, and then distilled under nitrogen (not pre-saturated). I am indebted to Mr. C. A. Watson of Hopkin and Williams Ltd. for this technique.

The 9,10-diphenylanthracene (Koch-Light Laboratories Ltd., scintillation grade; melting-point 250–252 °C, literature value[8] 245–247 °C) contained (as examined by high-performance liquid chromatography) no fluorescent impurity at a level greater than 1 part per 10^4 at excitation and emission wavelengths of 266 and 425 nm, respectively. An impurity giving a weak absorbance peak at 254 nm was eliminated when the sample was evacuated at 0.1 Torr for 30 min at 100 °C.

Quantum Yields

These are determined essentially according to Parker[9] from measurements at 260 nm on deoxygenated cyclohexane solutions, with reference to a value of 0.86 for 9,10-diphenylanthracene in the same solvent.[10]

Standard Comparison Procedure

The weighed sample is dissolved in cyclohexane to give a concentration of 20 μg ml^{-1}, and the ultraviolet absorption spectrum recorded at a 1-cm path length. If necessary, the solution is diluted until its absorbance does not exceed 0.10, with reference to a solvent blank, at wavelengths greater than 260 nm. For weakly absorbing samples more concentrated solutions may be needed, but this event is unusual. The solutions are photolabile,[11,12] and should therefore be stored in darkness and used within about 3 h. Spectral changes occur even in refrigerated samples on prolonged storage.

The solution is transferred into the 5-mm spectrofluorimeter cell and purged with solvent pre-saturated nitrogen until there is no further increase in the fluorescence monitored with excitation and emission wavelengths of 286 and 310 nm, respectively. With a nitrogen flow-rate of 10 ml min^{-1} the purging is usually complete within 1–2 min. Three synchronous fluorescence spectra are now recorded, all at the same sensitivity and in the corrected excitation mode, with separations between excitation and emission wavelengths of 34, 20 and 7 nm, and with both excitation and emission half-band widths of 3 nm. The excitation wavelength range of each spectrum is 230–430 nm.

Numerical Representation of Spectral Features

The features, listed in Table I and in most instances indicated in the spectra in Fig. 1, are measured on the ultraviolet absorption and fluorescence spectra of the samples. Those features that are fluorescence intensity values are corrected for any contribution from the solvent blank, and for inner filter effects as described under Discussion. They are also corrected for variations from the true response of the emission detection side of the spectrofluorimeter by multiplication with the ratio, at the relevant wavelength, of the response obtained when the spectrum of the xenon source lamp is synchronously scanned with the emission corrector accessory in operation to the response obtained without the corrector; Fig. 1(*c*) shows the effect of the correction. The intensity values (*i.e.*, features 2–6, 9–14 and 16–20) are finally normalised to give a value for their sum of 10^4.

Discussion

Spectrofluorimetric Parameters and Presentation

The luminescence of petroleum products is due mainly to complex mixtures of heavily alkylated aromatic hydrocarbons and sulphur heterocycles.[13] In some materials mixtures

of unsubstituted or only lightly alkylated polynuclear aromatic hydrocarbons are also present in sufficient amounts to provide the basis of earlier fluorescence fingerprinting techniques,[14] but the progress made in the chromatography of this group of compounds has made their characterisation, prior to separation, by luminescence techniques largely redundant.

The total fluorescence of petroleums has been represented in three dimensions in the form of spectral contour diagrams by Freegarde *et al.*[15] and by a number of other workers more recently. The diagrams are dominated by a common structure,[16] however, and usually exhibit only a strong single or occasionally double peak, varying in wavelength between samples to some extent, together with various sub-features, which are largely obscured. An example from a crude oil is shown in Fig. 2. The relatively characterless appearance of the diagrams is reflected in the two-dimensional excitation or emission spectra more commonly used for sample comparisons.

TABLE I

DEFINITIONS OF SPECTRAL FEATURES

Feature	Spectrum*	Definition†
1	UV	(*Ad*, feature 2)/(sample conc., g ml^{-1})
2	D34	(F_0 max. before 282 nm) − (F_0 at solvent base line)
3	D34	(F_0 max. 292–302 nm) − (F_0 min. 282–292 nm)
4	D34	(F_0 at 313 nm) − (F_0 min. 302–308 nm)
5	D34	(F_0 at 324 nm) − (F_0 at solvent base line)
6	D34	(F_0 max. 343–356 nm) − (F_0 min. 336–343 nm)
7	D34	Wavelength of feature 2
8	D34	Wavelength of feature 6
9	D20	(F_0 max. 266–277 nm) − (F_0 at solvent base line)
10	D20	(F_0 at 278 nm) − (F_0 min. 278–283 nm)
11	D20	(F_0 max. 283–298 nm) − (F_0 min. 278–283 nm)
12	D20	(F_0 max. 303–312 nm) − (F_0 min. 298–303 nm)
13	D20	(F_0 max. 323–331 nm) − (F_0 min. 316–323 nm)
14	D20	(F_0 max. 379–385 nm) − (F_0 min. 370–379 nm)
15	D20	Wavelength of feature 11
16	D7	(F_0 max. 270–284 nm) − (F_0 at solvent base line)
17	D7	(F_0 max. 298–312 nm) − (F_0 min. 284–298 nm)
18	D7	(F_0 max. 324–334 nm) − (F_0 min. 312–324 nm)
19	D7	(F_0 max. 351–359 nm) − (F_0 at solvent base line)
20	D7	(F_0 max. 388–398 nm) − (F_0 at solvent base line)
21	D34, UV	(Feature 2) (*Ad*, feature 2)$^{-1}$ (D34 solvent Raman)$^{-1}$
22	D34, UV	(Feature 1) (feature 21) × 10^{-3}

* D34, D20 and D7 are synchronous fluorescence spectra with scanning intervals of 34, 20 and 7 nm.

† The *Ad* value used in the calculation of features 2 and 21 is from the inner filter correction of feature 2, as described in the text. Each F_0 value is a fluorescence intensity, corrected for inner filter and instrumental effects as described in the text, at a specific point or at a point of maximum or minimum intensity within the excitation wavelength range indicated.

Increased differentiation between samples may be obtained from difference contour diagrams,[12,16] but the synchronous fluorescence technique[17] presented in this form offers greater selectivity. Thus, as in Fig. 3, the contours representing fluorescence intensity can be plotted as a function of excitation wavelength (horizontal axis) and of the interval between emission and excitation wavelengths (vertical axis). This diagram has been constructed from a set of synchronous fluorescence spectra covering the area in Fig. 2 within the pair of diagonal lines that indicate sections of the diagram corresponding to synchronous spectral emission - excitation intervals of 5 and 45 nm. Clearly, even with allowance for the greater number of spectra taken over the pertinent area, much more detail is apparent in the synchronous diagram. Although Talmi *et al.*[18] and Weiner[19] have observed that a two-dimensional synchronous spectrum represents a diagonal section through a conventional contour diagram, the increased detail seen in synchronous contour diagrams has not been pointed out before.

As Vo-Dinh has noted,[20] whether the synchronous spectra are considered as excitation or emission spectra is an arbitrary matter, because when either the excitation or the emission

wavelength is specified, the other is defined by the scanning interval. It is, however, important to specify as in Fig. 3 whether the spectra are being considered as excitation or emission spectra for the purpose of recording them, otherwise ambiguities arise. In this paper all of the synchronous spectra are plotted with reference to their excitation wavelengths.

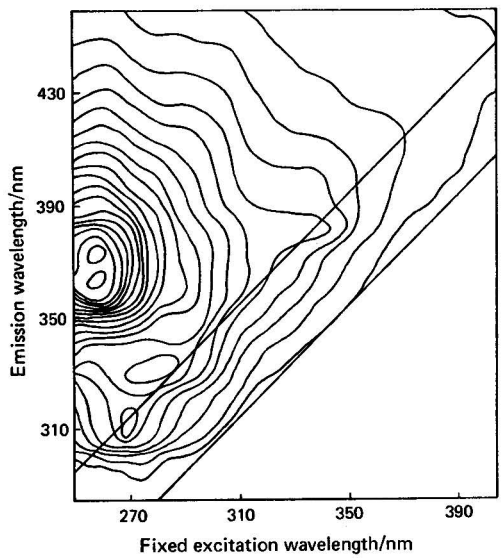

Fig. 2. Conventional fluorescence contour diagram of a crude oil in deoxygenated cyclohexane. The contours are at 5% intervals of intensity, increasing as the excitation wavelength decreases. The intensities are excitation-corrected only. The pair of diagonal lines indicate the sections represented in synchronous spectra with scanning intervals of 5 (lowermost) and 45 nm.

Although rapid techniques are now available[18,21,22] that could be developed for the computerised production of synchronous fluorescence contour diagrams or related forms of display, it is doubtful whether the construction of complete diagrams could be justified on every occasion when comparisons are to be made. From the considerable variety of samples that have been examined in the present connection, it is apparent that the same groups of fluorescent compounds, which vary only slightly in their peak wavelengths (presumably according to their degree and nature of alkylation), are always present even though their amounts are sometimes very small. Hence, comparisons between samples are essentially quantitative comparisons between the magnitude of, rather than the presence or absence of, specific features. Such comparisons are most easily made between two-dimensional spectra where intensities are displayed continuously rather than incrementally as in the synchronous contour diagrams. Most of the features evident in the diagrams can be collected economically in a small number of two-dimensional spectra obtained with a restricted number of scanning intervals, *e.g.*, in the region of 10, 20 and 30 nm in Fig. 3. The intervals of 7, 20 and 34 nm actually chosen for routine use (see Experimental) are a compromise dictated by the requirements of resolution, sensitivity and numbers of features found for a variety of samples. Although most of the features used are relative fluorescence intensities, the performance of some fluorescence wavelength and ultraviolet absorbance measurements as discriminators will also be examined elsewhere in Part II.[23] All of the features are defined in Table I, and many of them are shown in the spectra in Fig. 1. Some further examples of the spectra, and their ability to discriminate between similar samples, are shown in Fig. 4. These spectra are from three samples of the same brand of a common motor oil, each of which is clearly

differentiated from the other two (replicate spectra of each sample are superimposable on one another).

When the fluorescent components of a mixture are adequately known, their synchronous fluorescence characteristics can be predicted, which avoids the development of analytical techniques likely to generate spurious data.[24] The empirical approach adopted here, which is analagous to Weiner's[19] except that the basis is a synchronous contour diagram, is necessitated by the still poorly known nature of petroleum fluorescence, and inevitably produces some redundant data, as the results obtained show.[23] However, the results also show that features of importance are revealed at each of the three scanning intervals. Previous applications of the technique in this field have tended to be restricted to single scanning intervals.[2,25–28]

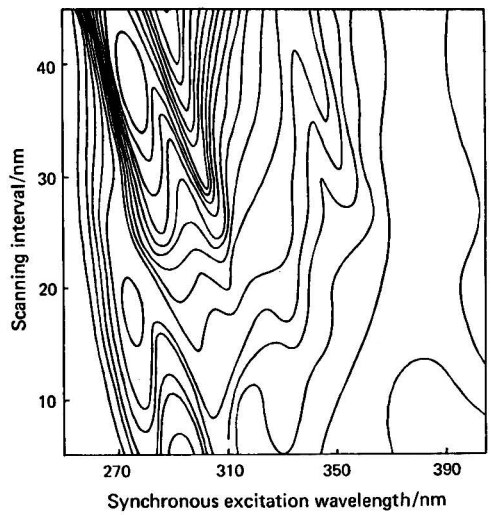

Fig. 3. Synchronous contour diagram of the area between the diagonal lines in Fig. 2. Otherwise the experimental conditions and the solution are the same as in Fig. 2. The contours are at 5% intervals of intensity, maximising at the 280-nm region with intervals of about 40 nm.

Other Techniques

Several other luminescence techniques are available by which further detail may be obtained. These include low-temperature luminescence spectroscopy and the presentation of spectra in a derivative form.

The increased detail present in the fixed excitation and emission luminescence spectra of petroleums when recorded at 77 K rather than at ambient temperatures was reported by Parker[29] and, more recently, by Fortier and Eastwood.[30] Their spectra show contributions due to both fluorescence and phosphorescence. Under these conditions more detail is obtained by the synchronous technique, as first reported for extender oil in tyre prints,[31] and subsequently for heavy oils by Eastwood *et al.*[2] However, for samples that are undifferentiated at ambient temperatures by the technique described under Experimental, the increased differentiation obtained at 77 K of samples commonly of interest in this work is usually small. For instance, the synchronous spectra of the three common motor oils of different manufacture shown in Fig. 5, which are not differentiated at ambient temperatures (broken lines spectra; *a*, *b* and *c*), remain undifferentiated at 77 K (full lines) despite the increases in detail both at this scanning interval (20 nm) and at others from which further detail is apparent, as in the 10-nm spectrum shown in Fig. 5(*d*).

At the usual synchronous scanning intervals (*e.g.*, less than 100 nm) no phosphorescence is detected from these samples because of the typically large shift between their phosphorescence

excitation and emission spectra. Larger intervals are generally required in phosphorimetric applications.[32] When, under the appropriate conditions, the phosphorescence or both the phosphorescence and the fluorescence are plotted under either fixed or synchronous conditions, still no differentiation between the samples in Fig. 5 is obtained. Of these various techniques, the excitation spectrum of the total phosphorescence (passed through the emission monochromator set at zero order, and separated from the fluorescence and the scattered excitation by a rotating can phosphoroscope) exhibits the greatest number of features. A spectrum typical of the three samples in Fig. 5 is shown in Fig. 6 (broken line). Even a very different sample, such as that giving the full-line spectra in Fig. 4, is now poorly differentiated, as the spectrum in Fig. 6 (full line) shows. The corresponding phosphorescence emission spectra compare closely to the spectrum of a motor oil given by Parker,[29] and do not vary significantly between any of the samples used to obtain Figs. 5 and 6.

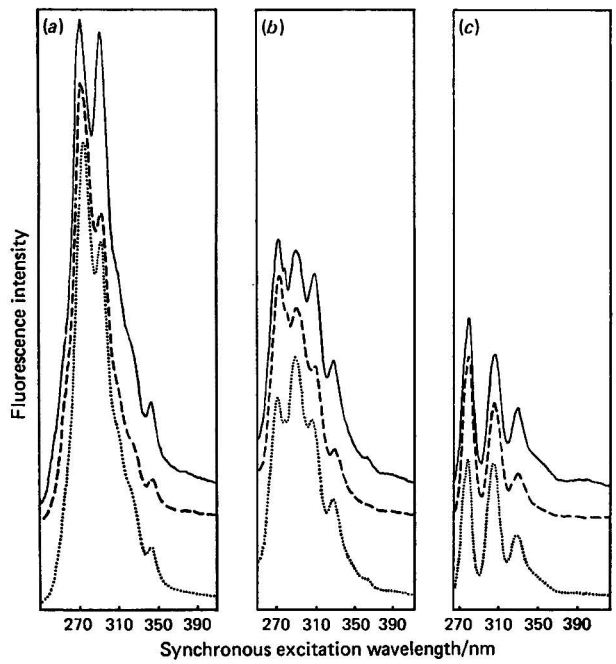

Fig. 4. Synchronous fluorescence spectra at intervals of (*a*) 34, (*b*) 20 and (*c*) 7 nm of a solution (20 μg ml^{-1}) in deoxygenated cyclohexane of each of three different samples of the same brand of an unused motor lubricant.

The apparent inconsistency between these results and the value placed on low-temperature synchronous luminescence spectroscopy by Eastwood *et al.*[2] must be due in part to differences in the nature of their samples. However, the inconsistency must also be partly due to the increased detail obtained at ambient temperatures in this work by the deoxygenation of the solutions (see below). If 77 K spectra are compared with the spectra of aerated solutions at ambient temperatures,[30] then part of the increased detail at 77 K is due to a reduction in oxygen quenching that is not seen if the point of reference, at ambient temperatures, is a deoxygenated solution.

Examples of the increased detail displayed by the first-derivative presentation of fixed excitation - emission spectra have been given by Green and O'Haver[3] and Eastwood *et al.*[2] Increased detail may be similarly obtained from synchronous spectra, as John and Soutar have suggested.[27] An example, in the second-derivative form, is given in Fig. 7, where features are now seen that are barely discernible in the original spectrum, shown by the

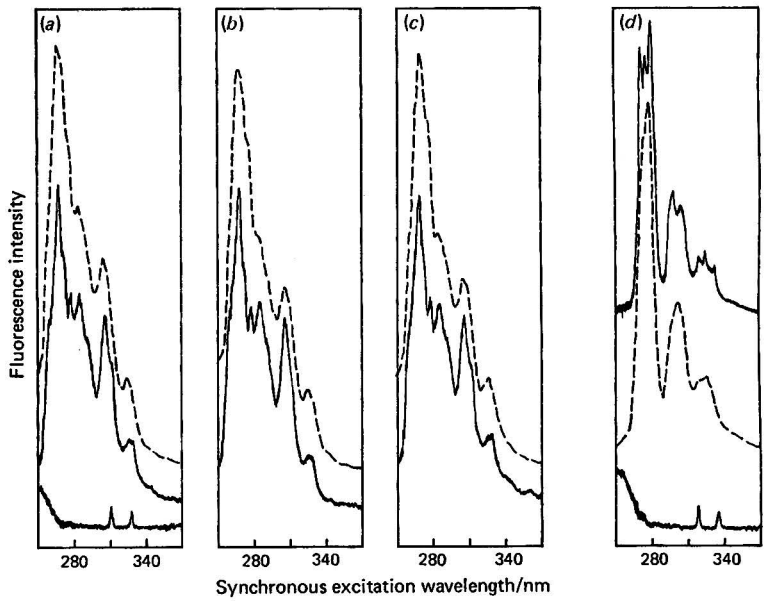

Fig. 5. Synchronous fluorescence spectra at ambient temperatures (broken line) and 77 K (full line) of three motor oils of different manufacture at a scanning interval of 20 nm [(*a*), (*b*) and (*c*)]. The samples are 20 μg ml^{-1} in methylcyclohexane, which is deoxygenated for the ambient temperature spectra. Spectra (*d*) are from the solution used for (*c*) but scanned at 10 nm. The two solvent spectra [(*a*) and (*d*)] show narrow peaks originating in the silica Dewar vessel used.

broken line. (Both spectra are uncorrected, to avoid a large contribution from the instrument's spectrum corrector accessory to the background noise that is otherwise present in the derivative spectra.) However, it is again found that the technique does not significantly increase discrimination amongst samples not already discriminated by the standard technique, because the increased detail given by the derivative presentation is not matched by a level of reproducibility appropriate to the close comparison of relative concentrations. In the future, this position might be changed by the application of the techniques recently described by Talsky *et al.*[33] for the production of higher order derivatives of absorbance spectra by means of an analogue computer unit.

Frank[34] has introduced a "fluorescence maxima profile" technique, which presents the fluorescence of a sample as an envelope excited at all possible wavelengths. Some examples were also given by Tanacredi.[35] Although the profiles may be of value in the classification of samples,[35] the close comparison of samples by the technique is unlikely to afford much discrimination.

Deoxygenation Effects

Deoxygenation of a solution of a petroleum derivative results in a substantial increase in fluorescence intensity.[4] The effect is illustrated in Fig. 8, where all of the spectra (of a solution of a motor oil) have been plotted at the same sensitivity. Deoxygenation (full-line spectra) not only increases the over-all intensity of the fluorescence, but affects the features present in the spectra differentially. Some features are barely detectable prior to the deoxygenation.

The ratio of fluorescence intensity in aerated to deoxygenated cyclohexane solution for a variety of defined features (Table I, Fig. 1) is listed in Table II. The values quoted are from six different samples in whose spectra the features are strongly represented. The results emphasise that the maximum spectral detail cannot be obtained from petroleum

samples at ambient temperatures without deoxygenation. Indeed, one feature (12) that is found to be a valuable discriminator[23] is almost completely lost in aerated solutions.

Although at 77 K the effect of oxygen quenching on the spectra is negligible, precise comparison of emission intensities between samples is less readily made and, as before, there is little increase in differentiation. In certain instances increased differentiation is made available by the selective application of quenching effects, but this is best reserved for specific circumstances.[4] In any event, quenching effects can only be assessed with reference to unquenched spectra.

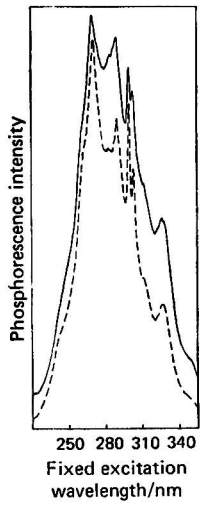

Fig. 6. Phosphorescence excitation spectra (uncorrected) at 77 K of solutions in methylcyclohexane. The full-line spectrum is from the same sample as the full-line spectra in Fig. 4. The broken-line spectrum is from the same sample as the spectra in Fig. 5(*a*).

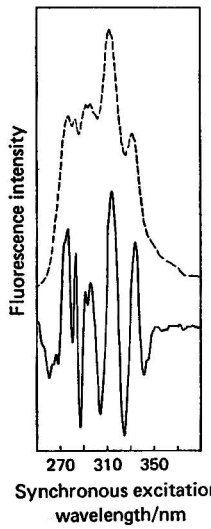

Fig. 7. Synchronous fluorescence spectra (uncorrected) at an interval of 20 nm of a motor oil (broken line) and the corresponding second derivative spectrum (full line).

Solvents

The spectra are not significantly varied by the use of different saturated hydrocarbon solvents (hexane, 2,2,4-trimethylpentane, cyclohexane, methylcyclohexane and the petroleum fraction boiling at 40–60 °C). Methylcyclohexane[30] and the petroleum fraction form glasses at 77 K and are useful, therefore, for low-temperature spectroscopy. Cyclohexane is used in most of the present work, at ambient temperatures, mainly because of its availability at a reasonable cost in a relatively pure state. Its strong Raman peak does not interfere at the sensitivities generally used, but interfering fluorescent impurities are normally present, both

TABLE II

FLUORESCENCE INTENSITIES OF PETROLEUM PRODUCTS IN AERATED RELATIVE TO DEOXYGENATED CYCLOHEXANE

Feature*	2	3	5	6	9	11	12	13	16	17	18	19	20
Mean† ..	0.67	0.11	0.62	<0.05	0.53	0.84	0.09	0.55	0.51	0.73	0.67	0.66	0.67
Standard deviation ..	0.02	0.06	0.04	—	0.04	0.11	0.11	0.08	0.06	0.02	0.06	0.08	0.05

* Defined in Table I.
† Six samples of different oils.

in this and in the other solvents, whatever their specified purity. The usual techniques of purification over silica gel, aluminium oxide and aluminium oxide loaded with silver nitrate[36] have proved inferior to the activated charcoal technique (see Experimental).

Unless quenching effects are to be deliberately employed, the presence of halogenated (except fluorinated) solvents is avoided, either for extraction of samples from substrates or for spectroscopy: the presence of 1% by volume of carbon tetrachloride in cyclohexane, for instance, reduces the emission intensity of petroleum products in the region of 300 nm to approximately 10% of the unquenched value. Quenching and spectral modification are also induced by chloroform and, to some extent, by dichloromethane,[4] although Keizer and Gordon[37] have reported otherwise.

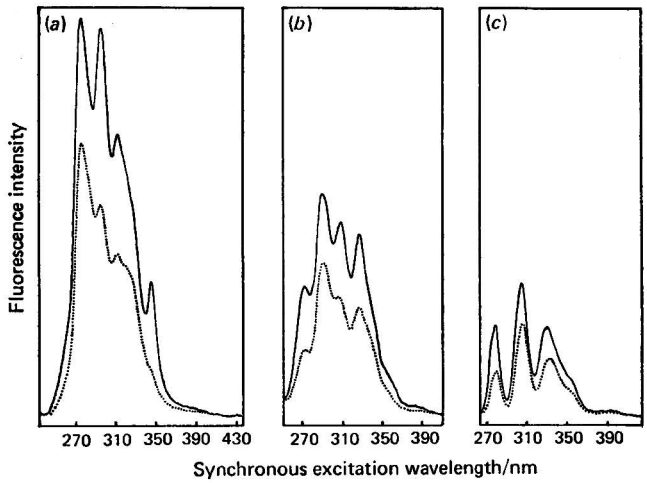

Fig. 8. Synchronous fluorescence spectra (excitation-corrected) at intervals of (*a*) 34, (*b*) 20 and (*c*) 7 nm of a 20 μg ml^{-1} solution of a motor oil in cyclohexane at ambient temperature. The dotted-line spectra are from an aerated solution; the full-line spectra are from a deoxygenated solution. All of the spectra were run at the same instrumental sensitivity.

Concentration and Inner Filter Effects

The ultraviolet absorbance spectra of petroleum products, of which some examples are shown in Fig. 9, generally rise gradually from the visible region to a plateau or weakly resolved peak at 250–265 nm. Thereafter, absorbances rise steeply, sometimes to give a peak at about 230 nm. The $E^{1\%}_{1\,cm}$ values at 260 nm found for the range of samples encountered to date in forensic work vary between 5 and 500, and are useful discriminators. (They are, incidentally, strongly correlated with the refractive indices of the samples, which have also been used as discriminators.[38] Thus, for seven samples in which $E^{1\%}_{1\,cm}$ at 260 nm varies from 10 to 222, and $n_D^{20\,°C}$ varies from 1.474 to 1.513, the correlation coefficient is 0.90.)

Many of the ultraviolet-absorbing compounds in these complex mixtures are not significantly fluorescent—evidently, from their phosphorescence, many undergo inter-system crossing to triplet states after excitation. Thus, the quantum yield of fluorescence emission (see Experimental) in the region of the excitation maximum at 260 nm of a sample of a crude oil, an extender oil, a motor oil and a light lubricating oil, all in deoxygenated cyclohexane, are found to be 0.16, 0.19, 0.13 and 0.13, respectively. Further, the wavelength range of the fluorescence emission is widely dispersed with a width at half peak height corresponding to about 5000 cm^{-1}, *e.g.*, 340–410 nm. For single compounds a value as great as 4000 cm^{-1} is unusual. Hence, relatively strongly absorbing solutions must be used if a sample signal strength is to be obtained that is sufficiently in excess of solvent blanks and the spectrometer noise level for the reproducible measurement of spectral features.

At the concentrations used, commonly 20 μg ml^{-1} (see Experimental), the absorbances of the solutions in the region of the shortest wavelength features may be as great as, *e.g.*, 0.09 and 0.05 per centimetre of path length, at excitation and emission wavelengths of 270 and 290 nm, respectively. If the provisional assumption is made that the observed fluorescence intensity, F, at a given wavelength can be corrected for inner filter effects[39] by the factor 10^{Ad}, *i.e.*, $F_0 = 10^{Ad}F$, where F_0 is the corrected intensity, A is the sum of the absorbances per centimetre of path length at the excitation and emission wavelengths and d is the cell depth at which the fluorescence is monitored; then, in the present example (0.5-cm cell, $d = 0.25$ cm; $A = 0.09 + 0.05$), $F_0 = 1.084F$.

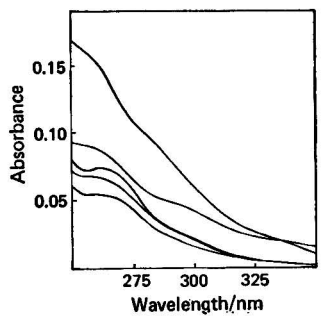

Fig. 9. Ultraviolet absorption spectra (with reference to a solvent blank), (1-cm path length) of solutions (20 μg ml^{-1}) in cyclohexane of three different motor oils, a crude oil and a grease, in order of increasing absorbance at 275 nm.

However, this factor is an underestimate. For non-polar molecules in weakly interacting solvents, and if excited-state intermolecular processes may be neglected, the corrected fluorescence intensity should vary linearly with the concentration (C) of the sample: $F_0 = KC = 10^{Ad}F$, where K is a constant. Hence, $\log(F/C) = \log K - Ad$. In agreement with this, experimental plots of $\log(F/C)$ against A are linear over the range up to $A = 1.0$, but with slopes more negative than the nominal value of $-d$. An empirical value of d, derived from a variety of features and samples, is therefore used in the calculation of the correction factors.

If the fluorescence intensities from solutions of a sample at two concentrations, indicated by subscripts 1 and 2, are compared:

$$F_{0,1}/F_{0,2} = C_1/C_2 = (F_1/F_2)\ (10^{A_1 d}/10^{A_2 d})$$

When the ratio of the concentrations is 10, and if the Beer - Lambert law applies (this has been confirmed), $A_1 - A_2 = 0.9A_1$, and then

$$d = [1 - \log(F_1/F_2)]\ (0.9A_1)^{-1}$$

In this way, ten features of four typical samples at concentrations of 100 and 10 μg ml^{-1} gave 33 values of d (in seven instances the fluorescence intensity and the absorbances were too weak to be measured reliably), distributed with a mean and standard deviation of 0.353 and 0.060 cm, respectively. There is no significant variation of d with either feature or sample, and therefore the mean value is adopted for the calculation of the correction factors. For the above example, the factor is now 1.12.

Other correction procedures are available that make allowance, for example, for the finite variation in the depth within the cell from which the measured fluorescence is collected,[40]

and for the spectral distribution of the light emerging from a monochromator,[41] but these require that the optical path lengths in the cell be defined. Although the initial assumption in the present treatment may be poorly justified theoretically, the errors involved are written off into the empirical *d*, for which an absolute value is unnecessary.

In earlier studies of petroleum luminescence, consideration of the strong inner filter effects sometimes evident has often been avoided by the use of standard concentrations. Consequently, whether the variations between samples in apparent fluorescence intensities are due to variations in the ultraviolet absorbances of the samples or in the concentrations of their fluorescent components cannot be determined. Hence, one set of discriminating features, from the ultraviolet absorbance spectrum, is lost; and another, the fluorescence intensities relative to a given amount of sample, is conditional on the equivalence of the inner filter effects in the samples concerned. Petroleums have been discriminated from one another partly on the basis of the changes caused to their fluorescence spectra by increases in concentration,[42] but these changes are due to inner filter effects, which yield information more readily obtained by ultraviolet spectroscopy.

Conclusion

In Part II of this work[23] the standard fluorescence comparison procedure (see Experimental) will be applied to a collection of samples, principally from case work, chosen to represent the range of high relative molecular mass petroleum products likely to be encountered as contact traces, with the object of assessing the efficiency of the various defined features as discriminators. Given these results, the development of computerised data collection and processing should facilitate the subsequent work on the establishment of evidential significance in specific circumstances.

As in an earlier paper,[14] it is strongly emphasised that for the characterisation of small amounts of petroleum derivatives there are other techniques available, such as infrared spectroscopy and those based on chromatography, that give results uncorrelated with the fluorescence results. Therefore, where the sample size permits, all of these techniques must be applied if evidential significance is, in general, to be optimised, even though on certain occasions fluorescence techniques have yielded evidence inaccessible by any other means.

I very gratefully acknowledge the help, at the points already indicated, of Mr. D. A. Collins and Mr. C. A. Watson.

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Received *April* 26*th*, 1979
Accepted *September* 11*th*, 1979

Determination of Tetramethyl- and Tetraethyllead Vapours in Air Following Collection on a Glass-fibre - Iodised Carbon Filter Disc

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Methods are described for the determination of tetraalkyllead vapour in air. The related sampling procedure operates efficiently over a wide range of lead-in-air concentrations, flow-rates and sampling periods. It is also effective under extreme conditions of humidity, can be applied in the presence of gasoline vapour and is especially convenient for personal monitoring or for use in the field.

The air sample is passed through a glass-fibre - iodised carbon filter on which tetraalkyllead compounds are collected. Lead is then extracted from the filter and converted into the inorganic state by treatment with iodine solution, followed by colorimetric determination as lead dithizonate using a commercially available test kit. In an alternative procedure involving the use of nitric acid - bromine reagent for extraction, the lead is determined by atomic-absorption spectrophotometry with electrothermal atomisation.

The efficiency of the methods has been confirmed using laboratory prepared air samples containing 5–400 $\mu g\ m^{-3}$ of lead.

Keywords: *Tetramethyllead determination*; *tetraethyllead determination*; *air analysis*

Tetraethyllead (TEL) and tetramethyllead (TML) are manufactured on a large scale for use as antiknock additives to gasoline. Owing to the toxicity of organolead compounds it is necessary to monitor exposure to tetraalkyllead during manufacturing operations, distribution and use. The current threshold limit value - time-weighted average (TLV - TWA) is 100 $\mu g\ m^{-3}$ for TEL and 150 $\mu g\ m^{-3}$ for TML.

Several of the published methods for the determination of tetraalkyllead compounds in air have been based on dry-sampling procedures. Snyder and Henderson[1] described a simplified field method in which iodine crystals were used as a collector. In this method a standard volume of sample was taken at a fixed sampling rate. A later method by Snyder[2] involved collection of tetraalkyllead on active carbon over a period of up to 4 d but it was necessary to carry out a lengthy extraction procedure with nitric acid - perchloric acid prior to the lead determination. Coker[3] also used a dry-sampling system, with an 8-h sampling period, and completed the determination by atomic-absorption spectrophotometry, as did Harrison *et al.*[4] as the final stage of their method in which the sample was collected over a period of 30 min on a gas - liquid chromatographic column cooled by dry-ice. Laveskog[5] established a method involving the use of gas chromatography linked with mass spectrometry, the sampling time being 15 min.

This paper describes a sampling procedure that has a wide range of applications and is suitable for use in monitoring concentrations of tetraalkyllead in air both below and above the TLV. Sampling can take place over periods from 10 min to 24 h with appropriate sampling rates from 1 to 40 $l\ min^{-1}$. The tetraalkyllead compounds are collected on a glass-fibre - iodised carbon filter. Only a simple treatment of the filter is necessary to remove the collected lead and the determination can be completed by a variety of analytical techniques.

When laboratory facilities are not available the tetraalkyllead is extracted from the glass-fibre - iodised carbon filter with iodine solution. The concentration of lead in the resulting solution is determined spectrophotometrically using dithizone (diphenylthiocarbazone). The reagents are commercially available as a lead-in-air kit. When laboratory facilities are available an alternative procedure can be used. The tetraalkyllead is extracted from the glass-fibre - iodised carbon filter with nitric acid - bromine solution and the con-

centration of lead in the resulting solution is determined by atomic-absorption spectrophotometry using electrothermal atomisation. The determination can also be completed by anodic-stripping voltammetry or by a spectrophotometric method.

Experimental

Instruments

Pitman lead-in-air analyser, Model 705. Supplied by D. A. Pitman Ltd., Weybridge, Surrey.

Perkin-Elmer 300 *S atomic-absorption spectrophotometer with an HGA-*76 *carbon furnace atomiser accessory.*

Reagents

For use with the Pitman lead-in-air analyser

The following ampouled reagents (D. A. Pitman Ltd.) are supplied for use with the lead-in-air analyser.

Iodine, 0.2 N *in methanol.*

Iodine, 2 N *aqueous solution in potassium iodide.*

Solution A. A lead-free aqueous solution of potassium cyanide, sodium sulphite, ammonium citrate and ammonium hydroxide contained in two ampoules.

Dithizone in chloroform, 40 mg l^{-1}.

Caution—Chloroform is a suspected carcinogen and inhalation of the vapour should be avoided.

Details of the preparation of lead-free reagent solutions were given by Snyder and Henderson.[1]

For use in alternative methods of analysis

Potassium iodide solution, 25% *m/V.* Dissolve 500 g of potassium iodide in about 1 l of distilled water. Make the solution slightly alkaline by the dropwise addition of ammonia solution (sp. gr. 0.880) and then de-lead it by shaking it with successive 50-ml portions of 20 mg l^{-1} of dithizone in chloroform solution until the green colour of the dithizone solution remains unchanged. After separation of the organic phase make the solution slightly acidic by the dropwise addition of dilute nitric acid and then wash it with chloroform in order to remove any dissolved dithizone. Separate the chloroform layer and make up the volume of the remaining solution to 2 l with distilled water.

Iodine monochloride stock solution, 1.0 M. Mix 445 ml of de-leaded 25% *m/V* potassium iodide solution with 445 ml of analytical-reagent grade concentrated hydrochloric acid. Slowly, with cooling, add 75 g of analytical-reagent grade potassium iodate, stirring the solution until all of the free iodine has re-dissolved to give a clear orange - red solution; dilute the solution to 1 l with distilled water.

Iodine monochloride solution, 0.1 M. Dilute 1 volume of iodine monochloride stock solution with 9 volumes of distilled water.

Caution—

1. Rubber bungs must not be used to stopper vessels containing iodine monochloride solution.
2. Iodine monochloride will react with ammonium ions under certain conditions to yield nitrogen triiodide, which has violently explosive properties. It is important, therefore, that ammonia and ammonium salts should be excluded from solutions containing iodine monochloride, except when an excess of reducing agent is also present.

Nitric acid solution, 50% *V/V.* Dilute 1 volume of Aristar-grade concentrated nitric acid with 1 volume of distilled water.

Nitric acid solution, 1% *V/V.* Dilute 1 volume of Aristar-grade concentrated nitric acid with 99 volumes of distilled water.

Bromine, Aristar grade.

Standard inorganic lead solution, 100 μg ml^{-1}. Dissolve 0.160 g of analytical-reagent grade lead nitrate in distilled water, add 10 ml of Aristar-grade concentrated nitric acid and make up the volume to 1 l with distilled water.

Standard inorganic lead solution, 0.2 μg ml^{-1}. Take 2 ml of 100 μg ml^{-1} standard inorganic lead solution, add 10 ml of Aristar-grade concentrated nitric acid and dilute to 1 l with distilled water.

Pre-treatment of the glass-fibre - carbon filters

Soak Whatman glass-fibre - carbon filters, grade ACG/B, 25 mm diameter, in 0.2 N methanolic iodine in a sealed container for 15 min. Remove the filters carefully using ivory-tipped forceps and allow them to dry in a desiccator for a minimum period of 2 h. This procedure may be carried out in the laboratory prior to use.

Blank determination and limit of detection

It is necessary to carry out several blank determinations as the lead content may vary, but normally it is less than 1 μg of lead per filter. The determination is carried out by the methods described later in the paper.

The method based on the Pitman analyser involved the use of a comparator disc. At the lower end of the disc a scale reading of 0.5 can be estimated with reasonable confidence. Blank levels using this method rarely exceed a disc reading of 0.5 (equivalent to 1 μg of lead). It was therefore considered acceptable to base the limit of detection on a disc reading of 0.5, which when expressed as a concentration of tetraalkyllead in air the detection limit is 8 μg m^{-3} for a 1-h sampling period at a sampling rate of 2 l min^{-1}.

When using the atomic-absorption method the limit of detection was calculated in the following way. Ten blank glass-fibre - iodised carbon filters were extracted and the solutions were diluted to 100 ml. Ten more blank glass-fibre - iodised carbon filters were spiked with 1 μg of lead, extracted and the solutions diluted to 100 ml so that the lead content was slightly above the blank value. Volumes of 20 μl of each of the sets of solutions were injected alternately into the furnace. The total lead content of each solution was calculated by reference to a calibration graph. The mean value obtained was 0.89 μg of lead. The lead found was calculated by subtracting the mean of two adjacent blanks from the blank + standard value. The mean value obtained for the lead found was 1.05 μg and the standard deviation was 0.2357 μg. The detection limit was considered to be twice the standard deviation. When expressed as a concentration of tetraalkyllead in air the detection limit is 4 μg m^{-3} for a 1-h sampling period at a sampling rate of 2 l min^{-1}.

Collection of the sample

Dismantle a Gelman 25 mm diameter, in-line filter holder and, using ivory-tipped forceps, place one of the treated carbon filters centrally on the mesh support. Re-assemble the holder and, using the minimum length of plastic tubing, connect the holder to the pump. Connect the other end of the holder to a tube flow meter, switch on the pump in a lead-free atmosphere and adjust the flow to the desired rate. Turn off the pump and disconnect the tube flow meter. Attach the pump and holder in the required position and sample for the required period. If the sampling period is greater than 30 min it is essential to check the sample volume using a dry-air pollution meter.

Analysis of the sample

The concentration of tetraalkyllead compounds collected on the treated glass-fibre - carbon filter can be determined by one of the following two methods.

Using the Pitman, Model 705, *lead-in-air analyser and reagents.* After completion of the sampling, dismantle the filter holder and, using ivory-tipped forceps, transfer the carbon filter into a midget impinger tube. Add 15 ml of 0.2 N iodine in methanol, stopper the tube and shake the contents for 1 min. Add 10 ml of 2.0 N iodine in aqueous potassium iodide solution and swirl the tube to mix the solution. Warm the solution to at least 27 °C and hold it at this temperature for a minimum of 5 min. Filter as much as possible of the iodine solution through a fine glass-wool plug into the comparator flask. Wash the flask and glass-wool with two 10-ml volumes of distilled water followed by three 5-ml volumes of distilled water. Add 35 ml of solution A to the comparator flask. Care should be taken not to allow any free iodine to remain around the neck of the flask. Stopper the flask and shake it until the iodine is completely decolorised. Pour 5 ml of chloroform into the vial containing 0.2 mg of dry dithizone and add this solution to the comparator flask. Vigorously shake the comparator flask for 30 s and allow the two layers of liquid to separate. If the lower layer is colourless or has a slight greenish tint, there is no lead present. If a red colour is obtained in the lower layer and the upper layer is orange - yellow, place the com-

parator flask in the Lovibond comparator and rotate the colour disc until a colour match is obtained.

NOTES—

1. If an orange or red - orange colour is obtained in the chloroform layer an incomplete reaction of organic lead with the iodine is indicated. The analysis must be repeated, making sure that the solution is heated to and held at a minimum temperature of 27 °C for at least 5 min to permit complete reaction of the lead compounds with the iodine.
2. If the upper layer does not appear yellow and the colour of the chloroform layer is darker than the deepest shade on the disc, insufficient dithizone is present and another portion of dithizone should be added. Shake the comparator flask for a further 30 s and obtain a colour match. If the colour is still too deep for matching then it will be necessary to repeat the test but running for a shorter length of time.

Carry out a blank determination using a treated glass-fibre - carbon filter.

Using atomic-absorption spectrophotometry. After completion of sampling, dismantle the filter holder and, using forceps, transfer the carbon filter into a 150-ml beaker. Add 10 ml of 50% *V/V* nitric acid and 1 ml of bromine. Cover the beaker with a watch-glass and warm the solution gently until all of the bromine has evaporated. Digest the solution on a hot-plate for 1 h, then evaporate the solution almost to dryness. Cool, add 10 ml of 1% *V/V* nitric acid to the residue and warm. Filter the solution through a Whatman No. 54 paper to remove any insoluble matter and dilute to 100 ml with 1% *V/V* nitric acid or to a suitable volume depending on the lead-in-air concentration, sampling rate or sampling time.

Inject 20 μl of the solution into the carbon furnace using the following conditions: drying, 10 s at 150 °C; ashing, 10 s at 490 °C; and atomisation, 4 s at 2100 °C.

Prepare a calibration graph using 0.2 μg ml^{-1} standard inorganic lead solution under the conditions specified above. The linear range of the calibration is 0–2 ng of lead injected but it is acceptable to use a range of 0–4 ng of lead injected.

Carry out a blank determination using a treated glass-fibre - carbon filter.

Comparison of results obtained by various analytical techniques

The method using the Pitman analyser and that applying atomic-absorption spectrophotometry with electrothermal atomisation are described in detail in the paper. However, at various times during the course of our investigations it was found convenient to complete the determination by anodic-stripping voltammetry or by a dithizone spectrophotometric method. A bulk stock of the solution used for extracting the lead from the carbon filter was prepared and spiked with amounts of lead consistent with the range of lead-in-air concentrations studied. This stock solution was analysed by the four methods used in order to check their comparability. The results are shown in Table I.

It was concluded that valid results are obtained from the various techniques described in this paper.

TABLE I

COMPARISON OF RESULTS OBTAINED BY VARIOUS ANALYTICAL TECHNIQUES

	Lead found/μg			
Lead added/μg	Pitman analyser	Electrothermal atomic absorption	Dithizone spectrophotometry	Anodic-stripping voltammetry
6	6	6.5	6.0	5.9
6	6	6.8	6.3	6.0
12	10	11.4	11.5	11.1
12	12	11.7	11.8	11.8
48	48	49.6	49.3	48.3
48	44	49.6	48.6	48.1

Removal of particulate matter

When particulate lead compounds are present in an air sample, part or all of this lead will be included with the tetraalkyllead determined. The extent to which the determination of the tetraalkyllead is affected will depend on the nature of the lead compounds present

and in particular on their solubility in the reagent solutions. When it is necessary to take account of particulate lead compounds the sampling procedure should be modified as follows.

Dismantle a Gelman 25 mm diameter open filter holder and, using forceps, place one 25 mm diameter, 0.8 μm, Millipore Type AA membrane filter centrally on the mesh support. Re-assemble the holder and, using PTFE tubing as a sleeve, make a direct connection to the inlet of the holder containing the treated carbon paper.

Loss of tetraalkyllead due to adsorption on particulate matter

It was considered necessary to check whether tetraalkyllead is adsorbed on particulate matter in situations where a pre-filter is used. Under sampling conditions relevant to our studies the volume of air taken is less than 1.5 m^3 and the amount of particulate matter collected from this size of sample is rarely sufficient to give a visible stain on the pre-filter, even in an industrial environment. Therefore, in order to obtain a suitable amount of particulate matter for the investigation a much larger volume of air (32 m^3) was taken. Several weighed samples of particulate matter were collected on a Millipore membrane filter and these were used to check for adsorption of tetraalkyllead as follows.

Air samples containing a range of concentrations of tetraalkyllead were prepared by a diffusion method.[6] The sample was passed through a previously prepared particulate filter followed by a glass-fibre - iodised carbon filter at a rate of 2 l min^{-1} for a period of 1 h. A duplicate air sample was passed through a glass-fibre - iodised carbon filter only. The tetraalkyllead collected on both glass-fibre - carbon filters was determined using atomic-absorption spectrophotometry as previously described. The results are shown in Table II.

The results indicated that the tetraalkyllead concentration was unchanged after passing the air sample through the pre-filter and it was concluded that there is no significant adsorption of tetraalkyllead on particulate matter.

TABLE II

LOSS OF TETRAALKYLLEAD DUE TO ADSORPTION ON PARTICULATE MATTER

Mass of solid matter collected on pre-filter/mg	Lead-in-air concentration determined from glass-fibre - iodised carbon filter alone/μg m^{-3}	Lead-in-air concentration determined from glass-fibre - iodised carbon filter connected in series with a pre-filter/μg m^{-3}
1.1	9	8
0.9	33	31
1.3	72	87
0.8	81	80
0.7	178	188
0.8	236	227

Collection efficiency of untreated glass-fibre - carbon filter

Air containing a range of concentrations of tetraalkyllead was prepared by a diffusion method.[6] For the TML in air stream, a range of dilute solutions of pure TML in toluene were used in the diffusion cell. With TEL, pure TEL was used and the temperature of the diffusion cell was varied in order to cover the required range of lead-in-air concentrations. The sample stream was passed through a glass-fibre - carbon filter at a rate of 2 l min^{-1} for a given period of time. A duplicate air sample was passed through two scrubbers containing 15 ml of 0.1 M iodine monochloride at the same time and at the same flow-rate as for the test sample. It had previously been shown[7] that the collection efficiency of this system was satisfactory. The tetraalkyllead collected in the iodine monochloride was determined by an atomic-absorption technique in conjunction with a classical separation using dithizone.[8]

The tetraalkyllead collected on a glass-fibre - carbon filter was compared with that collected by iodine monochloride solution. A series of trials were carried out using dry air and water-saturated air streams. The tetraalkyllead collected on the glass-fibre - carbon filter was extracted and determined using the Pitman lead-in-air analyser as previously described. The tetraalkyllead collected by iodine monochloride solution was determined as lead dithizonate using a spectrophotometric single-extraction mono-colour method after reduction of the excess of iodine monochloride with sodium sulphite solution. The results obtained are shown in Table III.

It was concluded that the collection efficiency for dry air streams was satisfactory but the presence of water vapour seriously impairs the lead collection efficiency of the glass-fibre - carbon filter.

TABLE III

COLLECTION EFFICIENCY OF GLASS-FIBRE - CARBON FILTER AT A SAMPLING RATE OF 2 l min^{-1} FOR A PERIOD OF 1 h

Alkyllead present	Condition of air stream	Lead-in-air concentration determined from glass-fibre - carbon filter/ $\mu g\ m^{-3}$	Lead-in-air concentration determined from iodine monochloride/ $\mu g\ m^{-3}$	Lead collected by glass-fibre - carbon filter, %
TML	Dry	200	220	91
	Dry	150	170	88
	Dry	67	70	96
	Dry	67	74	91
	Water saturated	33	247	13
	Water saturated	25	168	15
	Water saturated	17	116	15
	Water saturated	17	116	15
TEL	Dry	233	251	93
	Dry	150	163	92
	Dry	67	69	97
	Dry	50	54	93
	Water saturated	133	205	65
	Water saturated	133	190	70
	Water saturated	42	55	76
	Water saturated	33	44	75

Application to water-saturated air streams containing tetraalkyllead

Attempts to remove water vapour selectively from sample streams by means of desiccating agents were unsuccessful, and means were therefore sought of minimising the effect of water vapour by modifying the collection system. The following treatment gave good results. The glass-fibre - carbon filters were treated with 0.2 N methanolic iodine solution and then allowed to dry in a lead-free atmosphere.

The tetraalkyllead collected on a pre-treated glass-fibre - carbon filter was compared with that collected by iodine monochloride solution. A series of trials were carried out using dry air and water-saturated air streams. The range of the method was extended to sampling periods of 7 h. The tetraalkyllead collected on the glass-fibre - carbon filter was extracted and determined using the Pitman lead-in-air analyser as previously described. The tetraalkyllead collected by iodine monochloride solution was determined as lead dithizonate using a spectrophotometric single-extraction mono-colour method after reduction of the excess of iodine monochloride with sodium sulphite solution. When the sampling period was 7 h, the tetraalkyllead collected by both the glass-fibre - carbon filter and the iodine monochloride solution was determined by anodic-stripping voltammetry after conversion into the inorganic state. The results obtained are shown in Table IV.

It was concluded that the collection efficiency for both dry air and water-saturated air streams was satisfactory at tetraalkyllead in air concentrations up to 250 $\mu g\ m^{-3}$ of lead.

Collection of tetraalkyllead from air over 24-h sampling periods using pre-treated glass-fibre - carbon filter

It was decided to check that the sampling procedure was efficient for monitoring tetraalkyllead (TML and TEL) over a period of 24 h with a sampling rate of 1 l min^{-1}. The tetraalkyllead collected on a pre-treated glass-fibre - carbon filter was compared with that collected by iodine monochloride solution connected in series behind the filter. The tetraalkyllead collected on the glass-fibre - carbon filter was extracted and determined as lead dithizonate using a spectrophotometric single-extraction mono-colour method. The tetraalkyllead collected by iodine monochloride solution was also determined as lead dithizonate using a spectrophotometric single-extraction mono-colour method after reduction of the excess of iodine monochloride with sodium sulphite solution. A comparison of the results obtained is shown in Table V.

TABLE IV

COLLECTION EFFICIENCY OF GLASS-FIBRE - IODISED CARBON FILTER AT A SAMPLING RATE OF 2 l min^{-1}

Alkyllead present	Condition of air stream	Period of sampling/h	Lead-in-air concentration determination from glass-fibre - carbon filter/$\mu g\ m^{-3}$	Lead-in-air concentration determination from iodine monochloride/$\mu g\ m^{-3}$	Lead collected by glass-fibre - carbon filter, %
TML	Dry	1	200	203	99
	Dry	1	200	218	92
	Dry	1	58	63	92
	Dry	1	67	63	106
	Dry	7	173	183	95
	Water saturated	1	150	158	95
	Water saturated	1	150	168	89
	Water saturated	1	25	27	93
	Water saturated	1	50	57	88
	Water saturated	7	218	239	91
TEL	Dry	1	133	137	97
	Dry	1	200	193	104
	Dry	1	67	71	94
	Dry	1	67	71	94
	Dry	7	218	240	91
	Water saturated	1	200	206	97
	Water saturated	1	200	206	97
	Water saturated	1	33	34	97
	Water saturated	1	50	56	89
	Water saturated	7	202	199	102

It was concluded that the collection efficiency of the pre-treated glass-fibre - carbon filter was satisfactory for monitoring tetraalkyllead in air at concentrations up to 200 $\mu g\ m^{-3}$ of lead for periods up to 24 h.

TABLE V

COLLECTION EFFICIENCY OF GLASS FIBRE - IODISED CARBON FILTER AT A SAMPLING RATE OF 1 l min^{-1} FOR A PERIOD OF 24 h

Lead-in-air concentration determined from glass-fibre - iodised carbon filter/$\mu g\ m^{-3}$	Lead collected in iodine monochloride connected in series behind the carbon filter/$\mu g\ m^{-3}$	Lead collected by glass-fibre - carbon filter, %
5	0.1	98
9	0.1	99
29	1	97
37	2	95
58	2	97
61	4	94
70	5	93
78	2	98
92	5	95
105	2	98
126	4	97
131	2	98
187	6	97

Collection of tetraalkyllead from air streams at high flow-rates

At sampling rates of 40 l min^{-1} it is not possible to pass a duplicate air stream through iodine monochloride solution as the collection efficiency of tetraalkyllead is low[8] at sampling rates above 4 l min^{-1}. To overcome this problem, a single air stream was prepared and passed through two pre-treated glass-fibre - carbon filters placed in series. The lead collected on the second glass-fibre - carbon filter was a measure of the collection efficiency of the first glass-fibre - carbon filter. The tetraalkyllead collected on both the glass-fibre - carbon filters was determined using atomic-absorption spectrophotometry as previously described. A comparison of the results obtained is shown in Table VI.

It was concluded that the collection efficiency of pre-treated glass-fibre - carbon filters was satisfactory at sampling rates up to 40 l min^{-1} for periods up to 2 h when the tetraalkyllead in air concentration did not exceed 400 $\mu g\ m^{-3}$ of lead.

TABLE VI

COLLECTION EFFICIENCY OF GLASS-FIBRE - IODISED CARBON FILTER AT A SAMPLING RATE OF 40 l min^{-1} FOR PERIODS UP TO 2 h

Alkyl lead present	Period of sampling/h	Lead-in-air concentration determined from 1st glass-fibre - iodised carbon filter/ $\mu g\ m^{-3}$	Lead-in-air concentration determined from 2nd glass-fibre - iodised carbon filter/ $\mu g\ m^{-3}$	Lead collected by glass-fibre - carbon filter, %
TML	0.5	29	0.4	99
	0.5	14	0.2	99
	2	23	0.1	100
	2	19	0.1	99
	2	116	0.7	99
	2	172	5	97
	2	333	24	93
	2	404	40	91
TEL	0.5	128	0.2	100
	0.5	196	0.1	100
	2	18	0.1	99
	2	23	0.1	100
	2	127	0.4	100
	2	175	1	99
	2	414	40	91
	2	526	81	87

Application to air streams containing gasoline vapour

An obvious application of the method would be the determination of tetraalkyllead in the atmosphere at oil-refinery sites. It was therefore necessary to check that the collection efficiency of the glass-fibre - iodised carbon filter is not impaired by the passage of gasoline vapour.

Air containing a range of concentrations of tetraalkyllead produced from four-star grade gasoline was prepared by a diffusion method as previously described. The air was passed through a glass-fibre - iodised carbon filter at a rate of 1–2 l min^{-1} for periods of up to 24 h. The tetraalkyllead collected on the glass-fibre - iodised carbon filter was compared with that collected by iodine monochloride connected in series behind the filter. The tetraalkyllead collected on the glass-fibre - iodised carbon filter was extracted and determined by anodic-stripping voltammetry. The tetraalkyllead collected in iodine monochloride solution was also determined by anodic-stripping voltammetry after conversion into the inorganic state. A comparison of the results obtained is shown in Table VII.

It was found that the collection efficiency of pre-treated glass-fibre - carbon filter was greater than 98% in the presence of gasoline vapour for tetraalkyllead in air concentrations up to 200 $\mu g\ m^{-3}$ of lead.

TABLE VII

COLLECTION EFFICIENCY OF GLASS-FIBRE - IODISED CARBON FILTER FROM AIR CONTAINING GASOLINE VAPOUR

Sampling rate/l min^{-1}	Period of sampling/h	Lead-in-air concentration determined from glass-fibre - iodised carbon filter/ $\mu g\ m^{-3}$	Lead collected in iodine monochloride connected in series behind the carbon filter/ $\mu g\ m^{-3}$	Lead collected by glass-fibre - carbon filter, %
2	1	207	2	99
2	1	200	<1	>99
1	24	106	<1	>99
1	24	204	<1	>99
40	2	127	2	98
40	2	136	2	99

Conclusion

The method developed can be used for the determination of tetraalkyllead in air over a wide range of concentrations, flow-rates and sampling periods. Collection efficiencies greater than 90% can be achieved for samples containing 5–400 $\mu g\ m^{-3}$ of lead by suitable choice of sampling period and flow-rate. The sampling equipment is especially convenient for personal monitoring or application in the field for periods of up to 24 h.

Acknowledgements are made to colleagues for their advice during the course of this work, and to the Associated Octel Company for permission to publish this paper.

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Received *May 30th*, 1979
Accepted *August 22nd*, 1979

Analyst, February, 1980, Vol. 105, pp. 119–124

Determination of Copper, Lead, Cadmium, Nickel and Cobalt in EDTA Extracts of Soil by Solvent Extraction and Graphite Furnace Atomic-absorption Spectrophotometry

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A procedure is described for the determination of copper, lead, cadmium, nickel and cobalt in EDTA extracts of soil and similar material. Diethylammonium diethyldithiocarbamate or ammonium tetramethylene dithiocarbamate metal complexes are extracted into xylene from the EDTA extracts, and metals are determined in the xylene phase by atomic-absorption spectrophotometry using a graphite furnace atomiser. The detection limits (concentrations in soil) are approximately copper 0.8, lead 0.3, cadmium 0.07, nickel 2.5 and cobalt 0.8 $\mu g\ g^{-1}$. These detection limits might be improved by at least a factor of 10. Iron, manganese, aluminium, calcium and zinc do not interfere in amounts likely to be found in extracts of natural or contaminated soils.

Keywords: Copper, lead, cadmium, cobalt and nickel determination; solvent extraction; atomic-absorption spectrophotometry; graphite furnace atomisation; soil analysis

Of several aqueous solutions prepared for extracting the "plant available" portion of a number of metallic trace elements, a buffered EDTA solution is one of the most commonly used. Various concentrations of EDTA, pH ranges, soil to solution ratios and extraction times have been employed in order to optimise the reproducibility and effectiveness of the extraction. The use of EDTA solutions in soil analysis has been reviewed by Borggaard.[1]

Trace metal concentrations in EDTA extracts of soil etc. are often in the nanograms per millilitre range, which makes atomic-absorption spectrophotometry with electrothermal atomisation a convenient method of determination. By introducing a solvent extraction step before the atomic-absorption measurement metal concentrations can be increased and matrix interferences decreased. Several trace elements form dithiocarbamate complexes, which can be quantitatively extracted into an organic solvent over a wide pH range.[2] In earlier work carbon tetrachloride or chloroform was often used as the solvent and the metal in question was determined by molecular spectrophotometry.[2] For the determination of several trace metals by flame atomic-absorption spectrophotometry extraction of dithiocarbamate complexes into 4-methylpentan-2-one has been employed.[3,4] Tjell and coworkers[5,6] used extraction with diethylammonium diethyldithiocarbamate (DDDC) in xylene from nitric acid solution prior to atomic-absorption spectrophotometry with electrothermal atomisation. Xylene is a convenient solvent as it is lighter than water, nearly insoluble in water, has good separation characteristics and is halogen free. It has been demonstrated that hydrocarbon halides, such as chloroform, may cause considerable loss of several elements during electrothermal atomisation.[7]

The purpose of this work was to demonstrate the usefulness of graphite furnace atomic-absorption spectrophotometry after solvent extraction with dithiocarbamates in xylene for the determination of copper, cadmium, lead, nickel and cobalt in EDTA extracts of soil and similar materials, and further to investigate the tolerance of the proposed method towards pH variations and interfering species.

* Presented at Euroanalysis III, Dublin, August, 1978.

Experimental

Reagents

All chemicals were of analytical-reagent grade, if not otherwise stated. Doubly de-ionised water direct from the de-ioniser proved satisfactory. All plastic apparatus and glass-ware are kept in nitric acid (1 + 1) when not being used.

Xylene. BDH; laboratory reagent, sulphur free.

Diethylammonium diethyldithiocarbamate (DDDC), 1.0% m/V solution in xylene.

Ammonium tetramethylene dithiocarbamate (ammonium pyrrolidine dithiocarbamate, APDC), 5.0% m/V solution in water. This solution is prepared daily and filtered before use.

Ethylenediaminetetraacetic acid, disodium salt (EDTA), 0.20 M solution.

Acetate buffer, pH 4.6. Acetic acid (1 mol) plus sodium acetate (1 mol) are dissolved in water and diluted to 1 l with water. Before use, 1 l of this solution is purified by extraction five times with 25 ml of 1% DDDC in xylene solution and five times with 25 ml of xylene.

Nitric acid, 1 + 3.

Sodium acetate solution, 2 M.

Standard solutions. Stock solutions of copper, lead, cadmium, nickel and cobalt, each containing 1000 μg ml^{-1} of one of these elements, are prepared from $CuSO_4.5H_2O$, $Pb(NO_3)_2$, $3CdSO_4.8H_2O$, $NiSO_4$, $(NH_4)_2SO_4.6H_2O$ and $CoSO_4.7H_2O$, respectively, and diluted to volume with 0.2 M nitric acid.

Stock solutions of iron, manganese, aluminium, calcium and zinc, each containing 1000 μg ml^{-1} of one of these elements, are prepared from metallic iron, $MnSO_4.H_2O$, metallic aluminium, $CaCO_3$ and $ZnSO_4.7H_2O$, respectively, and diluted to volume with hydrochloric acid so that the final solutions are 0.1 M in hydrochloric acid.

Apparatus

The atomic-absorption spectrophotometer used was a Perkin-Elmer, Model 303, instrument (without background corrector) equipped with Perkin-Elmer HGA-74 graphite cell, HGA-2100 controller and Model 56 recorder. Single-element hollow-cathode lamps were used as radiation sources.

Slits and wavelengths were set according to the instrument manual. The argon flow-rate through the graphite cell was 50 ml min^{-1}. When lead was determined the argon flow was interrupted during the atomisation stage.

Samples were injected with a Finnpipette (5–50 μl) or by means of the Perkin-Elmer AS-1 Auto-Sampling system (20 μl) using polyethylene sample cups. The atomisation conditions utilised are shown in Table I. For cadmium a relatively high charring temperature is necessary and for all of the elements determined a relatively long charring time is required in order to destroy the metal complexes.

TABLE I

ATOMISATION CONDITIONS

Condition	Metal				
	Cu	Pb	Cd	Ni	Co
Drying temperature/°C	175	175	175	175	175
Drying time/s	20	20	20	20	20/30
Charring temperature/°C	1000	500	500	1000	1100
Charring time/s	40	60	60	60	60
Atomisation temperature/°C	2500	2200	1800	2700	2700
Atomisation time/s	6	6	5	8	10
Wavelength/nm	324.7	283.3	228.8	232.0	240.7

Procedure

EDTA extraction

Before extraction the material is air dried at room temperature (20–24 °C), passed through a 2-mm sieve and further homogenised by hand grinding in an agate mortar. Then a 0.54-g amount is shaken for 24 h in an end-over-end shaker (30–40 rotations per minute) with 15.0 ml of acetate buffer, 15.0 ml of EDTA solution and 60.0 ml of water, and then filtered. The pH of this solution is about 4.6.

Solvent extraction and atomic-absorption spectrophotometric determination

Extraction is performed in 25-ml borosilicate test-tubes with standard ground-glass stoppers. All volumes are dispensed by means of Finnpipettes with disposable polyethylene tips. A 5–7-ml aliquot of aqueous phase and 1.00 ml of xylene phase are used throughout and the stoppered test-tube is shaken vigorously for 2–3 min by hand or by means of a reciprocating shaker. The two phases separate very rapidly. The metal content of the xylene phase is determined by graphite furnace atomic-absorption spectrophotometry on the same day (for cadmium, within 2 h). Samples should not be left in the polyethylene cups of the Auto-Sampler for more than 2 h and unknowns should be interspaced with standards in order to compensate for evaporation losses. It was found that 4% of the xylene would evaporate in 2 h.

For the determination of 15–150 ng of copper, 1–10 ng of cadmium or 5–100 ng of lead, 5.0 ml of EDTA extract (if less than 5 ml has to be used, acetate buffer is added until the total volume of the aqueous phase is 5 ml) plus 1.00 ml of 1% DDDC in xylene are shaken for 2 min.

For the determination of 50–800 ng of nickel, 5.0 ml of EDTA extract (or EDTA extract plus acetate buffer), 0.23 ml of nitric acid (1 + 3) decreasing the pH to about 3.8, 1.00 ml of 5% APDC in water and 1.00 ml of xylene are shaken for 3 min.

For the determination of 10–200 ng of cobalt the same procedure as for nickel is used, except that nitric acid is omitted.

Calibration procedure

In 25-ml test-tubes are prepared 5-ml volumes containing suitable amounts of metals within the ranges given above and acetate buffer, EDTA and nitric acid corresponding to unknowns. To these volumes are added 1.00 ml of 1% DDDC in xylene (copper, cadmium and lead) or 1.00 ml of 5% APDC in water plus 1.00 ml of xylene (cobalt and nickel). After shaking, the determination is carried out under the same conditions as for unknowns.

Standard-additions tests

To a suitable amount of an EDTA extract of soil (copper and lead 1 ml; cadmium 2 ml; cobalt 5 ml) were added 50 ng of copper, 25 ng of lead, 4 ng of cadmium or 10 ng of cobalt before solvent extraction. After measurement, the percentage recovery was determined.

Influence of pH on Metal Extraction

Copper, cadmium and lead

Amounts of nitric acid to give a range of pH values, acetate buffer, sodium acetate, EDTA and water to a volume of about 5 ml were added to amounts of diluted stock solutions containing 100 ng of copper, 50 ng of lead or 4 ng of cadmium. These solutions were prepared in duplicate. In one set of solutions pH was measured (pH 2–6) and to the other set of solutions was added 1.00 ml of 1% DDDC in xylene. Extraction and metal determination were performed as described above.

Nickel and cobalt

Two sets of solutions containing 500 ng of nickel or 200 ng of cobalt were prepared as above and 1 ml of 5% APDC was added (pH 3–6). The pH was measured as above. After addition of 1 ml of xylene and extraction, the metals were determined as described.

Interferences

Various amounts of the stock solutions of iron, manganese, aluminium, calcium and zinc were added to known amounts of the metals to be determined (copper 100 ng, lead 50 ng cadmium 4 ng, nickel 200 ng and cobalt 100 ng). Acetate buffer and EDTA solution corresponding to approximately 5 ml of EDTA extract were added. Then the determination was completed as described under Procedure.

Results and Discussion

It was found in preliminary experiments that copper, lead and cadmium could be extracted into the xylene phase by DDDC when the pH of the aqueous phase was about 4.6. The absorbances obtained were the same whether EDTA was present or not. With nickel and cobalt no absorbance was obtained when DDDC was used as extractant and the aqueous phase was 0.033 M EDTA solution and had a pH of 3–4.6. Using APDC as extractant cobalt gave the same absorbance with and without EDTA at pH 4.6. Using APDC nickel was extracted from an EDTA solution only when the pH was below 4.

Using accepted values of metal - EDTA complexation constants and side-reaction coefficients[8] and published metal - DDDC extraction constants,[9–12] it can be calculated that more than 99% of the copper, cadmium and lead present would be extracted into carbon tetrachloride or chloroform at pH 4.6 and the concentrations and volume ratios of EDTA and other reagents employed in the present study. Reported values of Ni - DDDC and Co - DDDC extraction constants vary considerably.[9,10,13] Employing the lowest values of the extraction constants, neither cobalt[9] nor nickel[13] should be extracted at pH 4.6.

Calculations based on published constants for the extraction of Ni - APDC[13] and Co - APDC[14] indicate that it should not be possible to extract the two metals into chloroform in the presence of EDTA at pH 4–4.6.

The distribution of metal - DDDC or metal - APDC complexes between an aqueous phase and xylene does not seem to have been investigated. Extraction constants for the extraction of Cu -, Pb -, Cd -, Ni - and Co - DDDC complexes into benzene have been found to be very similar to those for extraction into carbon tetrachloride or chloroform.[10]

The results obtained in this study agree with the results predicted by the above calculations for copper, cadmium and lead, but not for cobalt and nickel. It is not clear whether the reason for this is that there exist equilibria that have not been taken into account, or that there is a difference between xylene and the solvents that have been studied.

Influence of pH on extraction

It can be seen from Fig. 1 that the extraction of copper, cadmium and lead is complete within the pH range 1.5–6 using DDDC. Nickel is extracted only at pH <4 and cobalt at pH < 5 using APDC.

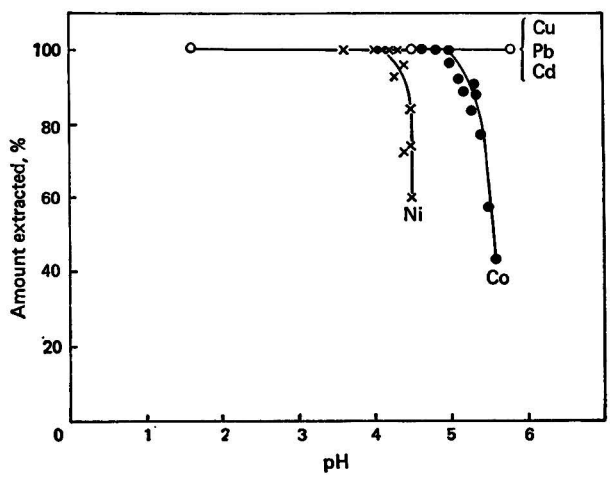

Fig. 1. Relative amounts of metals extracted from aqueous EDTA solution into xylene phase by DDDC (Cu, Pb, Cd) or APDC (Co, Ni) as a function of pH in the aqueous phase.

Interferences

The results of the interference tests are demonstrated in Table II. It should be noted that only in a few instances are the maximum amounts that can be tolerated shown. However, larger amounts than those tested are rarely encountered in EDTA extracts of soils, etc. When iron is present the xylene phase turns brown after extraction and the colour intensity increases with iron content. When about 1 mg of iron is present a precipitate is formed in the xylene phase, but even this amount did not interfere in the determination of cadmium. However, when determining nickel and cobalt the iron content may become close to the interference limit. With cobalt, up to 150 μg of iron may be present without interfering if the pH is decreased to 3.5.

TABLE II

INTERFERENCE TESTS

Amounts of common elements in the aqueous phase that did not interfere in the presence of the stated amounts of elements to be determined in 1 ml of xylene phase.

Amount of metal to be determined/μg	Amount of metal tolerated/μg				
	Fe	Mn	Al	Ca	Zn
Cu, 0.100	100	100	100	100	100
Pb, 0.050	100	100	100	100	100
Cd, 0.004	1000	100	100	1000	100
Ni, 0.200	100	100	1000	2000	100
Co, 0.100	50	200	1000	2000	50

Reproducibility and determination limits

When repeating the determination on the same xylene extract a deviation of 2% absorption or less between duplicates was considered satisfactory. It should be noted that the calibration graphs for copper, lead and cadmium intercepted the absorption axis at about 2–3% absorption. Calibration graphs for cobalt and nickel intercepted the absorption axis at a negative value.

Representative results from duplicate determinations on sewage sludge - soil mixtures, fly ash and soil are shown in Table III. A relative deviation of about 10% or less between duplicate EDTA extracts of solid sub-samples was considered satisfactory. Standard-additions tests demonstrated the absence of matrix interferences.

By using the procedure described, the following or larger amounts dissolvable in an EDTA solution from soil or similar samples can be determined: copper 0.8, lead 0.3, cadmium 0.07,

TABLE III

REPRESENTATIVE RESULTS

Metal	Determination*	Sludge - soil mixture/ μg g^{-1}	Fly ash/ μg g^{-1}	Soil/ μg g^{-1}	Standard-additions tests: Amount added to EDTA soil extract/ng	Standard-additions tests: Recovery, %
Cu ..	A	156, 157	22.7	9.79, 9.12	50	101
	B	166, 169	22.2	8.21, 8.70		
Pb ..	A	227, 216, 227	22.3	8.44, 8.78	25	104
	B	257, 244, 251	22.0	8.73, 8.21		
Cd ..	A	10.17, 9.10, 9.25, 9.85	0.96, 1.12	0.122, 0.098	4	94
	B	7.29, 6.90, 7.29, 6.90	1.00, 1.18	0.093, 0.086		
Ni ..	A	15.74, 14.75, 14.63	18.9			
	B	15.67, 15.75	19.7			
Co ..	A			0.32, 0.19	10	99
	B			0.27		

* For each metal, A represents replicate determinations on the same EDTA extracts and B represents replicate determinations on EDTA extracts of an identical sub-sample.

nickel 2.5 and cobalt 0.8 $\mu g\ g^{-1}$. It should be noted that the ratio of sample to EDTA solution (1:167) is not typical, normally a higher ratio (1:10) being employed. Therefore, it should be simple to lower the determination limits, although the possibility of interference, especially from iron, should not be overlooked. The determination limits might also be lowered by increasing the aqueous to organic volume ratio and/or by injecting a larger volume of xylene phase into the graphite furnace atomiser.

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Received *June 25th*, 1979
Accepted *August 30th*, 1979

Analyst, February, 1980, Vol. 105, pp. 125–130

Simultaneous Acid Extraction of Six Trace Metals from Fish Tissue by Hot-block Digestion and Determination by Atomic-absorption Spectrometry

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A simple and rapid digestion method is reported for the simultaneous acid extraction of chromium, copper, zinc, cadmium, nickel and lead from high-fat fish tissue. Samples are digested with nitric and sulphuric acids at 150 °C in a modified aluminium hot-block. The method is specially set up for fish sample sizes of up to 5 g, for low level detection of these elements. After digestion, acid extracts of the sample are analysed by direct flame atomic-absorption spectrometry for copper, zinc and chromium. The other three elements, cadmium, nickel and lead, are concentrated by chelation with ammonium tetramethylene dithiocarbamate followed by solvent extraction with isobutyl methyl ketone and determined by flame atomic-absorption spectrometry. The ease, rapidity and safety by which samples can be processed by this method make it suitable for the routine preparation of a large number of samples simultaneously.

Keywords: *Trace metal determination*; *fish analysis*; *hot-block digestion*; *atomic-absorption spectrometry*

In environmental pollution studies there is often the need for routine monitoring of toxic constituents in aquatic substrates. In large-scale studies, several trace parameters are often of interest in each sample obtained. Recently, our laboratory has been engaged in such a project, requiring methods for six trace elements (chromium, copper, zinc, cadmium, nickel and lead) in whole fish tissue. In the interest of speed, economy and simplicity, it would be a great advantage to be able to utilise a method that would simultaneously acid extract the six trace metals from the fish tissue, as well as making the digestion of a large number of samples possible.

Evans *et al.*[1] described a method for the simultaneous acid extraction of cadmium, nickel and lead from foodstuffs using Method (I)C of the Analytical Methods Committee.[2] It calls for digestion with nitric and sulphuric acids in Kjeldahl flasks. As specified[2] the method could be used with or without the addition of perchloric acid or hydrogen peroxide. As a simple and safe method is required for routine use, it is preferable to obtain a procedure that avoids the use of troublesome and possibly hazardous reagents, such as perchloric acid. The method indicated above has several limitations that make it unsatisfactory for the requirements of this study. The apparatus used does not lend itself to the simultaneous digestion of a large number of samples. In addition it was found that if high-fat samples were to be satisfactorily digested without the use of perchloric acid then the method had to be substantially modified.

Aluminium hot-blocks[3,4] have been used for the digestion of fish tissue at temperatures of 180 °C and over for the extraction of even a volatile element such as mercury. The possibility of using long test-tubes in the block produces a refluxing action during digestion and together with the ease of temperature control in the block makes it a very efficient and reproducible sample processing technique. The aluminium hot-block used by these workers is well suited to the simultaneous digestion of a large number of samples. Under the specified conditions[3,4] only 0.1–0.5 g of fish could be used. In this study, in order to achieve the required detection limits, it was necessary to use sample sizes of about 5 g, much larger than those used in previous hot-block digestions. It was therefore necessary to modify the aluminium hot-block as well as the digestion mixture used, in order to digest large samples satisfactorily and also to oxidise all the fat tissue.

In order that a parameter could be confidently determined in a sample, the analytical technique used must provide good sensitivity, and detection limits substantially lower than the level of the analyte in that substrate. The natural levels of chromium, copper and zinc are high enough that direct flame analysis of acid extracts, based on a 5-g sample size, provides satisfactory detection limits. For cadmium, nickel and lead, however, natural levels are so low that a further concentration step is required. In this study, it is shown that the digestion method adopted gives rise to solutions that are useful for chromium, copper and zinc determinations, and are also adaptable to the conventional ammonium tetramethylene dithiocarbamate (ammonium pyrrolidine dithiocarbamate) - isobutyl methyl ketone (APDC - MIBK) chelation - solvent extraction system of concentration. Under the scheme used in this study, all six trace elements could be determined with satisfactory detection limits from a single acid extract. The method outlined in this paper is simple, economical and safe and provides an effective means of multi-element determinations in the routine monitoring of a large number of fish samples.

Experimental

Apparatus

The aluminium hot-block is constructed as shown in Fig. 1. Calibrated digestion test-tubes are used in the block to digest the samples. The block is placed on a hot-plate capable of heating the block to a constant temperature of 150 °C. The temperature is monitored with a thermometer suspended in mineral oil placed in a tube in the block.

The aluminium block used in this study (Fig. 1) is a modification of that used by Bishop *et al.*[4] As large samples and large volumes of acid are used, it is essential to have as much of the digestion tube in the hot-block as possible. The depth of the holes was therefore changed from 1.5 to 2.5 in. This change allows the sample - acid mixture to be heated more uniformly.

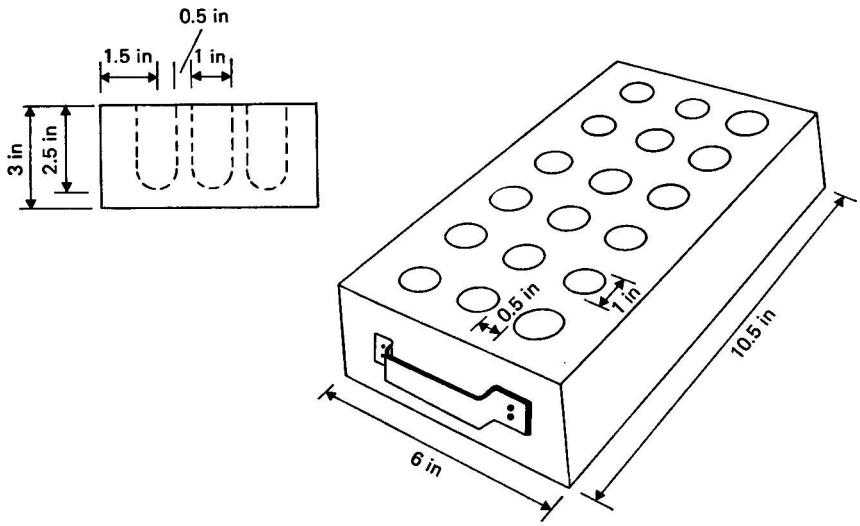

Fig. 1. Aluminium hot block.

All the metals were analysed using a Perkin-Elmer, Model 603, atomic-absorption spectrometer equipped with a triple-slot burner and deuterium background correction. An acid-resistant nebuliser was used for the direct analysis of chromium, copper and zinc from the acid extracts. A conventional nebuliser was used for the analyses of cadmium, nickel and lead after chelation and solvent extraction with the APDC - MIBK system. Deuterium background correction was used for the direct flame analyses.

Reagents

High-purity certified reagents were used for all analyses.

Nitric acid, 16 N.

Sulphuric acid, 36 N.

Ammonium tetramethylene dithiocarbamate (ammonium pyrrolidine dithiocarbamate, APDC) solution, 1% *m/V*.

Isobutyl methyl ketone (MIBK).

Hydrogen peroxide, 30% *V/V*.

Procedure

Digestion

Weigh about 5 g of fish into a calibrated digestion tube. Add 5 ml of nitric acid (16 N) and then 5 ml of sulphuric acid (36 N) to the sample. Allow the reaction to proceed, taking care not to allow any overflow of the sample. When the reaction slows, place the digestion tubes in the hot-block, heated to 60 °C, for 30 min. Remove the tubes from the hot-block and allow to cool for 5 min and then add another 10 ml of nitric acid. Return the tubes to the hot-block and increase the temperature, in steps, to 120 °C (the contents of the tubes should be boiling) until the liquid is about level with the top of the block. Increase the temperature to 150 °C. Remove the tubes when the samples go black, allow to cool for 5 min and add 1 ml of hydrogen peroxide (a vigorous reaction may occur). Return the tubes to the block. Repeat the hydrogen peroxide additions until the samples are clear. Remove the tubes and make up to 50 ml with de-ionised water when cool.

Analysis

Zinc, copper and chromium are analysed directly by flame atomic-absorption spectrometry. Standards must be treated in the same way as samples and carried through the whole procedure. It is very important that the standards contain the same amount of acid as the samples, especially sulphuric acid as the final solution contains 10% of this acid and its effect on the viscosity of the solution results in a significant suppression of sensitivity. This is strictly a physical effect and results in suppressions of sensitivity of 15, 10 and 25% for copper, zinc and chromium, respectively.

Nickel, lead and cadmium are concentrated by chelation - solvent extraction as follows. A volume of the sample digest (40 ml) is made up to 100 ml; 5 ml of the APDC solution and 5 ml of MIBK are added to the sample. The mixture is shaken vigorously for 5 min. Nickel, lead and cadmium are analysed in the MIBK phase by flame atomic-absorption spectrometry. Standards containing 1–100 $\mu g\ l^{-1}$ of each metal and 4% *V/V* sulphuric acid are also run.

Results and Discussion

Heavy metals are usually acid extracted from biological tissue by digestion with several different mixtures of nitric, sulphuric and perchloric acids. The most popular combinations are nitric acid - perchloric acid, nitric acid - sulphuric acid and nitric acid - perchloric acid - sulphuric acid.[5] Perchloric acid, as is well known, requires special precautions to be taken[6] and is undesirable for use in a simple routine method. Therefore, the mixture of choice would be nitric acid - sulphuric acid. Previously reported hot-block digestion methods have made use of a 1 + 4 nitric acid - sulphuric acid mixture to extract mercury from 0.1–0.5 g of fish tissue. As indicated earlier, in view of the low detection limits required in this study, 5-g sample sizes are required. The increased organic matter in such sample sizes makes the above acid mixture inadequate. Because most of the oxidative action of this mixture is due to the nitric acid, it was found that a modification of the mixture to 3 + 1 nitric acid - sulphuric acid effectively dissolved the whole sample under the conditions specified under Experimental. It is important to point out that the ease and rapidity of sample dissolution are a function of fat content. For example, fish fillet samples, which contain mainly protein tissue, dissolve completely at room temperature when left in contact with the acid mixture overnight. However, whole fish samples, which contain varying amounts of fat tissue, are only partially solubilised. For the dissolution of fat, heating at 150 °C was required. This

temperature together with the improved hot-block design described under Experimental, made the method satisfactory for the complete dissolution of 5 g of resistant high-fat tissue in the hot-block (Fig. 1).

As large volumes of acid are used for the dissolution of the large sample sizes used, the heating process in the aluminium block had to be controlled. It is important that the initial temperature be low to prevent the violent boiling-over of nitric acid. A ramp-heating step was used at 60 °C for 30 min, followed by 150 °C until dissolution was complete, in order to achieve the controlled heating. This arrangement proved very practical as a large number of fish samples could be treated with a minimum of attention. Under these conditions the nitric acid will boil continuously until it is completely evolved and only sulphuric acid is left. At this point, charring will definitely occur, owing to the large amount of carbon in these samples. While this is undesirable for volatile metals, such as mercury and selenium, because of the reducing environment set up, it has no detrimental effect on the relatively non-volatile elements of interest. Indeed, charring is advantageous in this method because it provides a means of breaking down resistant fat tissue. Sulphuric acid, being a strong dehydrating agent, effectively reduces the organic matter to carbon black, which can easily be cleared by the subsequent use of hydrogen peroxide. If charring is not allowed, by maintaining the nitric acid content in the digestion tube many more additions of acid would be required before all the organic matter is oxidised. By using a charring step no organic matter is left behind, which may interfere with the subsequent chelation - solvent extraction step. It should be noted that while charring, which occurs in dry-ashing methods, may cause the loss of some of the volatile elements such as cadmium and lead, charring in wet digestions has no such effect owing to the strong acid environment.

As indicated earlier, the natural levels of cadmium, nickel and lead are low enough that direct flame analysis of aqueous extracts does not provide satisfactory detection limits. The APDC - MIBK system, as outlined in the Water Quality Branch "Analytical Methods Manual,"[7] recommends the use of a buffer to adjust the pH of water samples to 3.5 for optimum simultaneous extraction of the six trace metals. It was found, however, that APDC complexes of cadmium, nickel and lead were distributed into MIBK with apparently little or no loss in solvent extraction efficiency when the buffering step was eliminated. This is fortunate as the sample extracts are highly acidic and buffering is not easily obtained, making it impractical for the desired routine method. Furthermore, much lower reagent blanks were obtained by eliminating the large amount of buffer needed.

TABLE I

DETECTION LIMITS TO BE EXPECTED FROM PROPOSED METHOD

Chromium, copper and zinc were analysed by direct aspiration and cadmium, nickel and lead were analysed by solvent extraction.

Metal	Detection limits/μg g^{-1}			Adopted detection limit/μg g^{-1}	Normal levels found in fish/μg g^{-1}
	A*	B†	C‡		
Cadmium	0.01	—	0.02	0.02	0.02–0.05
Nickel	0.01	0.05	0.04	0.05	0.05–0.19
Lead	0.01	—	0.08	0.1	0.1–0.2
Chromium	0.1	0.2	0.09	0.2	0.2–0.3
Copper	0.1	—	0.2	0.2	0.57–1.3
Zinc	0.1	—	0.2	0.2	0.9–1.5

* Lowest detectable signal.
† Concentration that gives a coefficient of variation of over 20%.
‡ Twice the standard deviation at the lowest detectable level.

Table I shows the practical detection limits that can be expected from the proposed method for the six trace metals of interest. As there is no universal agreement on a definition for detection limit, three commonly used methods of calculating it were used, as shown in Table I, and the highest of the three was used to obtain the recommended value. In this way the adopted value can more easily be reproduced by any laboratory. As shown in Table I these values are well below the required detection levels of the six trace elements found in whole fish tissue and thus the recommended technique becomes an effective tech-

TABLE II

STATISTICAL DATA FOR TISSUE FROM CONTAMINATED FISH

All data are based on 10 replicate determinations. Fish were exposed to cadmium, nickel and lead at increasing levels in fish tanks until mortality. Copper and zinc levels were naturally found levels while chromium was spiked into the rainbow trout homogenate (4) sample.

Tissue type	Cd		Ni		Pb		Cu		Zn		Cr	
	Mean/ $\mu g\ g^{-1}$	R.S.D.,* %	Mean/ $\mu g\ g^{-1}$	R.S.D.,* %	Mean/ $\mu g\ g^{-1}$	R.S.D.,* %	Mean/ $\mu g\ g^{-1}$	R.S.D.,* %	Mean/ $\mu g\ g^{-1}$	R.S.D.,* %	Mean/ $\mu g\ g^{-1}$	R.S.D.,* %
Rainbow trout homogenate (1)	0.10	12.1	0.16	15	0.92	4.3	0.6	13	10.9	4.8	—	—
Rainbow trout homogenate (2)	0.10	8.2	0.15	13	0.98	6.0	0.80	8.8	10.9	3.8	—	—
Rainbow trout homogenate (3)	0.11	9.1	0.20	17	0.94	8.3	0.53	6.4	11.8	9.3	—	—
Rainbow trout homogenate (4)	0.06	20	0.21	8.9	0.83	10	0.58	7.1	12.6	3.9	2.2	7.9
Fish liver	2.2	14	0.92	11	8.0	17	—	—	—	—	—	—
Fish kidney	7.1	9.2	1.9	5	36	11	—	—	—	—	—	—
Coho jack	—	—	—	—	—	—	1.06	5.4	24.6	6.7	0.21	15

* R.S.D. = relative standard deviation.

nique in determining trace metal levels for routine monitoring purposes. Table II shows some precision data for several types of fish tissue for the metals under study. The precision data for cadmium, nickel and lead are for unspiked fish tissue, but the fish had been subjected to metal-contaminated fish tanks for toxicity studies. Thus, the metal content was that which was ingested by the fish during their stay in these tanks. Therefore, these data give the true precision of recovery of the metals from the fish tissue, as compared with the reproducibility of spike experiments. The data for copper and zinc were natural levels found in fish and were therefore also incorporated into the fish tissue. Rainbow trout homogenate, sample 4, was spiked with chromium as no high levels of chromium were found in most of the fish analysed. The data in Table II show that the precision of the method depends not only on the level of metal but also on the nature of the sample.

TABLE III

RECOVERY AND PRECISION DATA FOR STANDARD REFERENCE MATERIALS

Data are based on 10 replicate determinations.

Material	Value	Concentration/$\mu g\ g^{-1}$ Cd	Ni	Pb	Cr	Cu	Zn
NBS* orchard leaves	Found	0.15 ± 0.05	1.18 ± 0.08	42 ± 1.7	2.7 ± 0.17	11.2 ± 0.18	25.3 ± 0.5
	Certified	0.11 ± 0.1	1.3 ± 0.2	45 ± 3	2.6 ± 0.3	12 ± 1	25 ± 3
NBS* bovine liver	Found	0.30 ± 0.07	—	0.28 ± 0.04	—	187 ± 2.3	131 ± 1.4
	Certified	0.27 ± 0.04	—	0.34 ± 0.08	0.088 ± 0.012	193 ± 10	130 ± 13
IAEA† fish flesh MA-A-2	Found	0.13 ± 0.04	1.34 ± 1.47	0.40 ± 0.04	2.92 ± 0.13	3.7 ± 0.46	32.4 ± 0.6
	Tentative by IAEA	0.16 ± 0.04	1.2 ± 0.2	0.7 ± 0.2	2.9 ± 0.6	4.6 ± 0.4	36 ± 3

* NBS = National Bureau of Standards.
† IAEA = International Atomic Energy Agency.

Recovery experiments were made by spiking fish samples with the six trace elements before digestion and then processing them in the same manner as the unspiked samples. Recoveries ranged from 90 to 110% in all instances and were classed as acceptable. To check further on the recovery of the method some certified standard reference materials were used to test the method. Table III shows the data for the mean of 10 replicate analyses for these reference materials. It can be seen that the data are satisfactory in relation to certified levels.

The proposed method is a simple, rapid, multi-element technique with satisfactory precision, accuracy and detection limits. As such, it provides an effective means for the routine monitoring of large numbers of samples.

The authors thank D. M. Whittle and P. V. Hodson for supplying the fish tissue.

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Received *August 20th*, 1979
Accepted *September 24th*, 1979

Analyst, February 1980, Vol. 105, pp. 131–138

Rapid Spectrophotometric Method for the Determination of Monofluorophosphate

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A method is described for the rapid spectrophotometric determination of orthophosphate in the presence of monofluorophosphate. Subsequent acid hydrolysis of monofluorophosphate enables this ion to be determined in the same sample. The technique is based on a single-reagent modification of the molybdophosphovanadate procedure. Using this method as described it is possible to determine up to 0.5 mg of phosphorus but this can be extended to 1.8 mg of phosphorus using an alternative procedure. Free phosphorus can be determined in the presence of up to a 50-fold excess of monofluorophosphate. The method is especially well suited for following hydrolysis reactions of monofluorophosphate.

Keywords: *Monofluorophosphate determination*; *orthophosphate*; *spectrophotometry*; *molybdophosphovanadate reagent*

The analytical determination of monofluorophosphate (MFP) or its ion (PO_3F^{2-}) presents a number of difficulties.[1] The ion hydrolyses rapidly in strongly acidic or strongly basic solutions, especially at elevated temperatures.[2–5]

The PO_3F^{2-} anion lacks characteristic properties of analytical importance and a method for its direct determination has yet to be reported.[5] Established methods for the determination of MFP generally adopt a separation step to remove contaminating species followed by hydrolysis of MFP to F^- and PO_4^{3-}, one or both of which is then determined.

Methods for determining MFP based on the spectrophotometric determination of orthophosphate have been developed. Ericsson[7,8] suggested that orthophosphate could conveniently be determined in the presence of MFP by an acid - molybdate reduction technique, provided that the hydrolysis reaction was controlled by rapid and accurately timed addition of reagents. Ingram[9] utilised the ascorbic acid reduction modification of the molybdenum blue procedure[10] to follow the acid hydrolysis of MFP. The method had the disadvantage of requiring frequent absorbance measurements. Also, the limit of determination for the method was 40 μg of phosphorus and, like all molybdenum blue methods, it was subject to a number of interferences. In a later work[6] Ingram reverted to a silver orthophosphate precipitation technique.

In the course of our work on the acid hydrolysis of MFP[1] it became necessary to determine the PO_4^{3-} in the presence of PO_3F^{2-} in a large number of samples. This paper describes a simple spectrophotometric procedure and is based on the single-reagent modification[11,12] of the molybdophosphovanadate technique.[13]

Experimental

Reagents

All reagents were of analytical-reagent grade except where stated. De-ionised distilled water was used throughout for the preparation of solutions.

Standard orthophosphate solution. Dissolve 2.197 g of potassium dihydrogen orthophosphate in water and dilute to 1 l (1 ml $\equiv$ 0.5 mg of phosphorus).

Acetate buffer solution. Add glacial acetic acid (57 ml) to water (443 ml). Adjust the pH to 5.2 using 10 M sodium hydroxide solution and dilute the solution to 1 l with water.

Molybdophosphovanadate spectrophotometric reagent. Dissolve 1.17 g of ammonium metavanadate in a mixture of water (400 ml) and 8 M perchloric acid (25 ml); then dilute the solution to 500 ml with water (solution A). Dissolve 35 g of ammonium molybdate tetrahydrate in water and dilute to 1 l (solution B). Prepare sodium sulphate solution (0.75 M) by dissolving 53 g of anhydrous sodium sulphate in water and diluting to 500 ml (solution C).

Final spectrophotometric reagent. Mix 70–72% perchloric acid, solution A, solution B and solution C (1 + 2 + 4 + 2) and store in a polythene bottle.

Apparatus

pH meter. Pye Dynacap.

Spectrophotometers. Perkin-Elmer, Model 124, ultraviolet - visible double-beam grating spectrophotometer connected to a Perkin-Elmer, Model 56, self-balancing multi-range recorder and fitted with a Hitachi 124-0319 10-mm thermostated cell holder. Also, a Varian, Model 654, ultraviolet - visible spectrophotometer with digital read-out calibrated to register concentrations directly and fitted with a thermostatic cell holder.

Procedure

Pipette the prepared reagent (10 ml) into a 25-ml calibrated flask. Dilute to about 20 ml with water. Cool in ice - water to about 4 °C. Add the sample and dilute to 25 ml. Measure the absorbance at 407 nm (or at the observed $\lambda_{max.}$). Prepare a calibration graph using known amounts of standard phosphorus solution containing between 0 and 0.5 mg of phosphorus (as KH_2PO_4). Treat unknown samples similarly and calculate the phosphorus concentration from the calibration graph.

Alternative Procedure

Samples containing up to 1.8 mg of phosphorus may be analysed with a resultant loss of sensitivity if all volumes are doubled and 50-ml calibrated flasks are used.

Results and Discussion

Sensitivity and Range

The Perkin-Elmer spectrophotometer used in much of this work was fitted with a pen recorder incorporating a variable voltage control. The range and sensitivity of the method using this equipment were, to a large extent, dependent on the recorder setting. When the alternative analytical procedure was used in conjunction with a recorder-voltage span of 50 mV, the calibration graph was linear for up to 1.8 mg of phosphorus and results were reproducible for up to at least 2.0 mg of phosphorus.

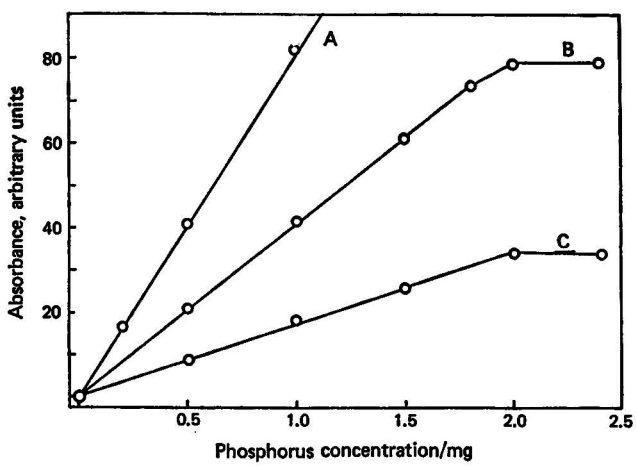

Fig. 1. Calibration graphs for the alternative molybdophosphovanadate procedure: 20 ml of reagent, 50 ml total volume, 1 cm path-length cells, absorbance measured at 407 nm. Recorder voltage range: A, 0–1 absorbance scale, 100 units = 20 mV; B, 0–2 absorbance scale, 100 units = 20 mV; and C, 0–2 absorbance scale, 100 units = 50 mV.

Under these conditions, however, the sensitivity was poor; a change in concentration of 0.5 mg of phosphorus produced a deflection of only 10% on the recorder.

Reduction of the recorder-voltage span to 20 mV produced a four-fold increase in sensitivity, but reduced the upper limit to 1.2 mg of phosphorus. Fig. 1 shows typical calibration graphs produced under these conditions.

When the recommended procedure was in use the linear range was reduced to 0–0.5 mg of phosphorus, but the sensitivity was greatly improved. Fig. 2 shows that under these conditions 0.5 mg of phosphorus represented a deflection of 86% and 0.05 mg of phosphorus a deflection of 9 $\pm$ 1%.

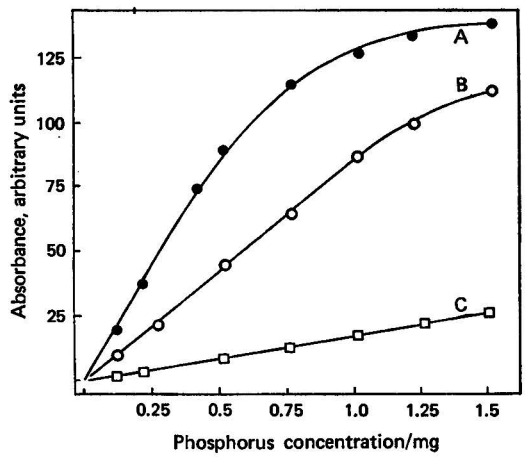

Fig. 2. Sensitivity of the molybdophosphovanadate procedure. Absorbance scales: A (recommended procedure), 100 units = 20 mV; B (alternative procedure), 100 units = 20 mV; and C (alternative procedure), 100 units = 50 mV.

Reproducibility

The reproducibility of the method was examined using 10 aliquots, each containing 0.25 mg of phosphorus as KH_2PO_4. The mean recovery was 0.2455 mg and the standard deviation was 0.0083 mg. This represents a recovery of 98.2% with a standard deviation of 3.33%.

Effect of pH

The measured pH of the final spectrophotometric solution was 0.4. The effect of increasing the pH was examined by preparing a series of molybdovanadate reagents identical with the standard reagents except that the perchloric acid concentration used in this preparation was adjusted to give reagents of varying pH. These were then used to prepare calibration graphs in the normal way using 20 ml of reagent in 50-ml calibrated flasks.

The standard reagent with a pH of 0.4 gave a linear graph for up to 1.5 mg of phosphorus, which was the highest phosphorus concentration used. As the pH was increased, the range over which linear calibration graphs were obtained was progressively reduced until at pH 5.0 it was linear only up to 0.6 mg of phosphorus. However, the sensitivity of the method remained essentially constant over the linear range.

Effect of Fluoride and Temperature on Colour Stability

Interference by fluoride ions would seriously restrict the value of the method for the determination of MFP in the presence of its hydrolysis products.

Possible interference was examined by adding aliquots of sodium fluoride solution to the reagents: (i) before the addition of phosphorus as KH_2PO_4; (ii) in admixture with phosphorus; and (iii) to the developed molybdophosphovanadate solution.

When the experiment was carried out at room temperature (18 °C) there was no interference from fluoride ion at concentrations of up to 0.02 M in the final solution (9.5 mg of fluoride), whatever the stage at which the fluoride was added.

However, if the colour was developed at elevated temperatures (60 °C) fluoride ion could only be tolerated up to a final concentration of 0.004 M. If added at a concentration of 0.02 M, fluoride exhibited a pronounced positive effect. This was a relatively constant interference equivalent to about 0.08 mg of phosphorus and bore no relationship to the amount of phosphorus present.

It is thought that this effect may be due to attack by hydrogen fluoride on the walls of the glass calibrated flasks used in the experiment when the acidic reagent is heated at 60 °C in the presence of fluoride.

Time Required for Full Colour Development

Kitson and Mellon[14] suggested that the presence of fluoride might increase the time required for full colour development.

The time needed for development of maximum colour intensity in the presence or absence of fluoride ion at a concentration of 0.004 M was noted for samples where the colour was developed inside or outside the spectrophotometer. In all instances this was found to be between 1.5 and 3 min. After 2.0 min 98% of the final absorbance was attained in five of the six samples studied. Thus, fluoride present at concentrations similar to those produced during hydrolysis of MFP did not affect the method.

Determination of Total Phosphorus in MFP

Weighed samples of MFP (0.1–3 g) were added to 50 ml of 1.0 M hydrochloric acid and heated at 60 °C for 30 min. Aliquots were analysed by the described method. Table I shows that the recovery of phosphorus was 98.25% of the theoretical value and the standard deviation was 1.36%.

TABLE I

RECOVERY OF PHOSPHORUS AFTER HYDROLYSIS OF MFP AT 60 °C

Mass of MFP/g	Volume of 1.0 M HCl/ml	Sample volume/ml	Number of of aliquots	Phosphorus found in aliquot (mean)/mg	Phosphorus found in MFP, %	Purity of MFP (calculated), %
0.360	50	0.20	4	0.300	20.83	96.76
0.720	50	0.10	4	0.305	21.18	98.38
1.440	50	0.05	4	0.304	21.11	98.06
2.880	50	0.05	4	0.620	21.53	100.0
					Mean	98.25
					Standard deviation	1.36

Comparison with Alternative Method

Four samples of the same batch of MFP were analysed by a method involving potentiometric titration after precipitation of silver orthophosphate.[12] This gave a recovery of phosphorus of 98.65% with a standard deviation of 0.81% (Table II).

TABLE II

RECOVERY OF PHOSPHORUS FROM HYDROLYSED MFP BY POTENTIOMETRIC TITRATION

Mass of MFP per 100 ml/g	Sample volume/ml	Titre of 0.0998 M NaCl solution/ml	Total phosphorus/mg		MFP purity, %
			Found	Calculated	
0.2251	20	9.2	47.44	48.46	97.9
0.2941	20	12.2	62.91	63.31	99.4
0.2760	20	11.3	58.26	59.42	98.0
0.2742	20	9.3	47.95	48.27	99.3
				Mean	98.65
				Standard deviation	0.81

Determination of Total Phosphorus in Mixed Solutions

A number of samples containing mixed MFP - KH_2PO_4 solutions were analysed by the method described above. Table III shows that the mean recovery of phosphorus from MFP for nine samples was 98.71% with a standard deviation of 2.40%.

TABLE III

RECOVERY OF PHOSPHORUS FROM MIXED MFP - ORTHOPHOSPHATE SAMPLES

Phosphorus added as—		Phosphorus found/mg		MFP purity, %
MFP/mg	KH_2PO_4/mg	Total	From MFP	
0.315	0	0.300	0.300	95.24
0.630	0	0.625	0.625	99.21
0.945	0	0.910	0.910	96.30
0.315	0.315	0.640	0.325	103.2
0.630	0.315	0.910	0.620	98.41
0.945	0.315	1.255	0.950	100.5
0.315	0.630	0.935	0.310	98.41
0.630	0.315	0.950	0.620	98.41
0	0.315	0.312		
			Mean	98.71
			Standard deviation	2.40

Purity of MFP

When results from all methods were considered, the mean content of the MFP used, expressed as a percentage of the theoretical value, was 98.6% with a standard deviation, determined over 16 samples, of 1.8%.

Determination of Free Orthophosphate in the Presence of MFP, Acetate Buffer Solution and Added Orthophosphate

When studying the rate of hydrolysis of MFP it is desirable to determine both liberated F^- and liberated PO_4^{3-} on the same sample. For reasons discussed elsewhere[1] it was found that the optimum buffer solution for this purpose was acetate buffer, pH 5.2. The effect of

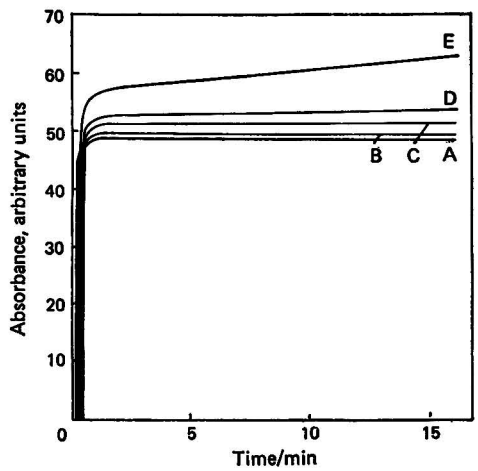

Fig. 3. Absorbance of PO_4^{3-} - MFP mixtures containing acetate buffer solution, over a period of time. A, 0.25 mg of P as KH_2PO_4; B, 0.25 mg of P as KH_2PO_4 + 1.44 mg of MFP; C, 0.25 mg of P as KH_2PO_4 + 4.32 mg of MFP; D, 0.25 mg of P as KH_2PO_4 + 7.2 mg of MFP; E, 0.25 mg of P as KH_2PO_4 + 14.4 mg of MFP.

TABLE IV

RECOVERY OF ADDED ORTHOPHOSPHATE WITH TIME IN PRESENCE OF MFP AND ACETATE BUFFER

Phosphorus added as KH_2PO_4/mg	MFP added/mg	Ratio of MFP to phosphorus	Phosphorus found/mg					
			2 min	4 min	5 min	8 min	10 min	15 min
0	1.44	—	0.0061	0.0061	0.0061	0.0061	0.0061	0.0070
0	2.88	—	0.0122	0.0122	0.0122	0.0122	0.0137	0.0171
0	4.32	—	0.0146	0.0159	0.0171	0.0183	0.0198	0.0243
0	7.20	—	0.0213	0.0240	0.0244	0.0298	0.0300	0.0396
0	14.40	—	0.0426	0.0482	0.0488	0.0610	0.0671	0.0850
0.25	0	—	0.252	0.250	0.250	0.250	0.250	0.250
0.25	1.44	5.76	0.257	0.255	0.255	0.255	0.255	0.255
0.25	2.88	8.64	0.257	0.257	0.258	0.258	0.258	0.258
0.25	4.32	17.28	0.266	0.266	0.266	0.266	0.266	0.266
0.25	7.20	28.80	0.273	0.274	0.274	0.276	0.278	0.280
0.25	14.40	57.60	0.297	0.299	0.302	0.307	0.313	0.323

TABLE V

RECOVERY OF ADDED MFP WITH TIME: EFFECT OF ADDED ORTHOPHOSPHATE

The results are for phosphorus (mg) as orthophosphate recovered from MFP.

Time/min	Orthophosphate addition*	Sodium monofluorophosphate added/mg					
		0	1.44	2.88	4.32	7.20	14.4
2	A	<0.005	0.0061	0.0122	0.0146	0.0213	0.0426
	B	<0.005	0.0050	0.007	0.0160	0.0230	0.0470
5	A	<0.005	0.0061	0.0122	0.0171	0.0244	0.0488
	B	<0.005	0.0050	0.008	0.0160	0.0240	0.0520
10	A	<0.005	0.0061	0.0137	0.0198	0.0300	0.0671
	B	<0.005	0.0050	0.008	0.0160	0.0280	0.0630
15	A	<0.005	0.0070	0.0171	0.0243	0.0396	0.0850
	B	<0.005	0.0050	0.008	0.0160	0.0300	0.0730

* A, No added orthophosphate; B, 0.25 mg of phosphorus added as KH_2PO_4 (this figure is given as P_{found} − 0.25 mg).

TABLE VI

RELEASE OF PHOSPHORUS FROM MFP WITH TIME: EFFECT OF ADDED ACETATE BUFFER

The results are for phosphorus (mg) found as orthophosphate.

Time/min	Acetate buffer addition*	Sodium monofluorophosphate added/mg					
		0	1.44	2.88	4.32	7.20	14.4
2	A	0.006	0.0061	0.0122	0.0146	0.0213	0.0426
	O	<0.005	0.000	0.0061	0.0110	0.0183	0.0426
5	A	0.006	0.0061	0.0122	0.0171	0.0244	0.0488
	O	<0.005	0.0030	0.0091	0.0171	0.0305	0.0610
10	A	0.006	0.0060	0.0137	0.0198	0.0300	0.0671
	O	<0.005	0.0043	0.0122	0.0238	0.0457	0.0976
15	A	0.006	0.0070	0.0171	0.0243	0.0396	0.0850
	O	<0.005	0.0061	0.0183	0.0305	0.0598	0.0134

* A, Acetate buffer (4 ml) added; O, no acetate buffer.

this buffer on the recovery of phosphorus from KH_2PO_4 and/or MFP solutions was examined by adding known amounts of phosphorus (as KH_2PO_4) and MFP to 4 ml of prepared acetate buffer solution and analysing aliquots of the resultant mixture. Table IV shows the recovery of added phosphorus from the reagent, as a function of time when different amounts of MFP were added. This experiment was conducted at room temperature (18 °C). Table V, similarly, shows the recovery of orthophosphate from MFP when additional amounts of phosphorus (as KH_2PO_4 solution) were added, and Table VI illustrates the effect of added acetate buffer on the release of phosphorus from MFP as a function of time.

These tables show that the addition of large amounts of acetate buffer solution inhibits the hydrolysis of MFP by the reagent at room temperature. The evidence from Tables IV and VI indicates that the acetate buffer used in this study contained about 0.001 5 mg ml^{-1} of free phosphorus. In the absence of MFP, full colour development occurred at the same rate whether or not acetate buffer was present. This suggests that acetate buffer does not inhibit the rate of colour development. The observed effect must therefore be due to inhibition of hydrolysis.

From Table V it can be seen that at high concentrations of MFP relative to phosphorus (above 4.32 mg of MFP in the presence of 0.25 mg of phosphorus) the earlier recoveries are higher than expected and also higher than those for the hydrolysis of MFP alone. However, even at an MFP to phosphorus ratio of 51.6:1, the hydrolysis reaction is inhibited by the addition of free orthophosphate.

Fig. 3 shows the absorbance of a number of PO_4^{3-} - MFP mixtures containing acetate buffer solution recorded over a period of 15 min. There is virtually no hydrolysis over this period unless the MFP to phosphorus ratio exceeds 17:1 and it is only at an MFP to phosphorus ratio of over 50:1 that reagent hydrolysis is a serious problem.

TABLE VII

REPRODUCIBILITY OF THE METHOD: FIRST BLIND TRIAL

Phosphorus added as—		Phosphorus found as orthophosphate/mg			MFP recovered, %
MFP/mg	KH_2PO_4/mg	Free phosphorus	Total phosphorus	Phosphorus from MFP	
0.158	0	0	0.165	0.165	104.4
0.158	0.158	0.158	0.315	0.150	94.9
0.210	0	0	0.215	0.215	102.4
0.052 5	0	0.01	0.055	0.054	102.9
0.079	0	0	0.075	0.075	94.9
0.210	0	0	0.215	0.215	102.4
0.157 5	0	0	0.150	0.150	95.2
0.210	0.158	0.158	0.360	0.205	97.6
0.105	0.105	0.105	0.205	0.100	95.2
0.263	0.052 5	0.052	0.312	0.260	98.9
0.236	0	0.01	0.241	0.240	101.7
0.105	0.210	0.215	0.315	0.100	95.2
0	0.315	0.315	0.315	0	—
0.157 5	0.157 5	0.165	0.315	0.150	95.2
0.210	0.105	0.110	0.310	0.200	95.2
0.263	0.052 5	0.055	0.315	0.260	98.9
0.236	0.079	0.075	0.315	0.240	101.7

	Orthophosphate, %	MFP, %
Mean recovery	100.98	98.54
Standard deviation ..	3.27	3.53

Reproducibility of the Method in MFP Determinations

Seven samples, each containing 0.157 5 mg of phosphorus as MFP, were analysed for free and total phosphorus by the methods described. The mean free phosphorus found was 0.007 mg and the mean total phosphorus was 0.155 7 $\pm$ 0.002 8 mg (which was 98.82% of the calculated result, standard deviation 1.82%).

Blind Trial

To establish the suitability of the method under actual experimental conditions two series of samples were analysed for free and total phosphorus. These samples contained unknown amounts of MFP and orthophosphate. Hydrolysis was carried out at 60 °C in polythene bottles. Tables VII and VIII show that there is good reproducibility between samples containing different proportions of phosphorus and MFP. The mean recoveries from these trials compared well with those where the amounts of MFP and phosphorus were known, but the standard deviation was higher [3.75% compared with 1.82% (see above) or 2.4% (see Table III)].

TABLE VIII

REPRODUCIBILITY OF THE METHOD: SECOND BLIND TRIAL

Phosphorus added as—			Phosphorus found as orthophosphate/mg		
MFP/mg	KH_2PO_4/mg	Total phosphorus/mg	Free phosphorus	Total phosphorus	Phosphorus from MFP
0.248	0.062	0.310	0.056	0.300	0.244
0	0.310	0.310	0.310	0.310	0
0.186	0.124	0.310	0.120	0.306	0.186
0.280	0.030	0.310	0.0275	0.300	0.273
0.062	0.248	0.310	0.250	0.310	0.060
0.155	0.155	0.310	0.145	0.305	0.160
0.093	0.217	0.310	0.210	0.310	0.100
0.031	0.279	0.310	0.265	0.310	0.045
0.217	0.093	0.310	0.080	0.300	0.220

	Orthophosphate, %	MFP, %
Mean recovery	98.4	100.7
Standard deviation ..	1.51	3.75

Conclusion

The described method offers a simple and rapid procedure for the determination of free orthophosphate in the presence of monofluorophosphate. Subsequent rapid acid hydrolysis allows MFP to be determined. It appears to offer many advantages over the molybdenum blue reduction technique, particularly in its relative freedom from interference by fluoride ion. The method is of particular value for following the acid hydrolysis of monofluorophosphate.[1]

The help and advice of Dr. Kjell Bjorvatn, Dental School, University of Bergen, Dr. Philos. Sverre Hauge, University of Bergen and Professor Dr. Philos. Tormod Mørch, Department of Pedodontics, University of Bergen, are greatly appreciated. Part of this work was undertaken at the University of Bergen. One of us (J.S.) expresses appreciation to the University of Bergen for the research facilities and financial aid provided.

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Received *May 22nd*, 1979
Accepted *August 8th*, 1979

Analyst, February 1980, Vol. 105, pp. 139–146

Automatic Determination of Boron (0.10–10.0 mg l^{-1}) in Raw and Waste Waters

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An automated method, employing azomethine-H in aqueous medium, for the determination of boron in raw waters and effluents is described. The method is capable of a limit of detection of 0.1 mg l^{-1} and the response is essentially linear to 4.0 mg l^{-1}, although a calibration graph is required above this level. A wide range of possible interferences was tested, but none proved of practical importance except sample colour, for which adequate correction is provided. Recovery after spiking a typical range of raw and waste waters was adequate.

Keywords: *Boron determination*; *AutoAnalyzer*; *water analysis*

The level of boron in raw waters has tended to increase in recent years, resulting from its greater use in cleansing materials and in industrial processes.[1] As sewage treatment does not significantly reduce this level the increase is passed to surface waters, which may then be utilised for crop irrigation or potable water supply. Many varieties of fruit can tolerate no more than 0.5 mg l^{-1} of boron[2] in irrigation water. The US Environmental Protection Agency[3] recommends limits of 0.75 mg l^{-1} for most fruits, 1.0 mg l^{-1} for most cereals, potatoes, peas and tomatoes and 2.0 mg l^{-1} for tolerant species including sugar beet, turnips and cabbage. The Anglian Water Authority[4] apply criteria such that the "maximum desirable" limit for crop irrigation is 0.5 mg l^{-1} and the "maximum permissible" limit is 1.0 mg l^{-1}. They also apply criteria of 0.8 and 1.0–1.2 mg l^{-1}, respectively, to potable water abstractions.

Several reagents have been developed for the routine determination of boron, but most require a final stage in concentrated sulphuric acid. Apart from the hazards involved in pumping the concentrated acid the procedures are difficult to automate, principally because of density effects resulting from small changes in acid concentration. These methods include the carminic acid procedure, which has been automated by the Water Pollution Research Laboratory.[5]

It has not been found possible to obtain the precision we require using this method. The curcumin procedure does not involve these dangers, but includes an evaporation stage unsuitable for automation. A method employing ferroin has been developed[6] involving extraction into chloroform, but this method suffers from interference by anionic detergents.

The principle adopted employs azomethine-H (see Fig. 1), a readily prepared condensation product of H-acid (8-aminonaphth-1-ol-3,6-disulphonic acid) and salicylaldehyde. This reagent is very sensitive to borates, forming, in aqueous medium, a yellow ion-associated compound by reversible reaction.

The method was developed by Shanina *et al.*[7] A manual procedure is employed by the Yorkshire Water Authority[8] and an automated method for the determination of boron in plant tissue has been developed by Basson *et al.*[9] This laboratory has further developed the procedure for use with the Technicon AutoAnalyzer 2 and to meet its own requirements of precision and sensitivity.

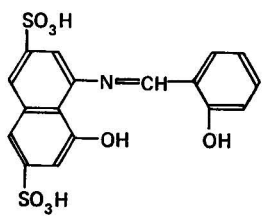

Fig. 1. Azomethine-H.

Experimental

Apparatus

A Technicon AutoAnalyzer 2 system is fitted with the manifold shown in Fig. 2. A sample cam of 30 h^{-1} is employed, with a 2:1 sample to wash water ratio; 410-nm wavelength filters are fitted.

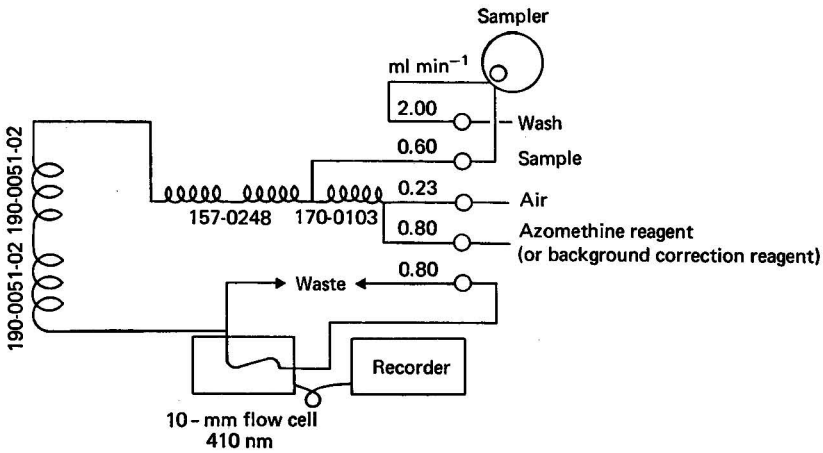

Fig. 2. Manifold arrangement for AutoAnalyzer 2.

Sample Preservation

The samples are filtered through Whatman grade C glass-fibre filter-papers immediately after collection and stored in completely filled polythene bottles at about 4 °C until required for analysis.

Reagents

All reagents were of analytical-reagent grade unless stated otherwise. De-ionised water was used in all solutions.

Boric acid. Dried to constant mass at 105 °C.

H-acid (*8-aminonaphth-1-ol-3,6-disulphonic acid, monosodium salt*). SynchemicA grade (Hopkin & Williams, Chadwell Heath, Essex).

Salicylaldehyde.

Concentrated hydrochloric acid. 36% *m/m* HCl.

Ethanol. Industrial methylated spirit.

L-*Ascorbic acid.*

EDTA (*ethylenediaminetetraacetic acid, disodium salt dihydrate*).

Acetic acid.

Ammonium acetate.

Brij concentrate. Dissolve 1 g of Brij 35 (polyoxyethylene lauryl ether) in water and dilute to 100 ml.

Potassium hydroxide solution, 10% *m/V.* Dissolve 10 g in water and dilute the solution to 100 ml.

Azomethine-H. Dissolve 18 g of H-acid in 1 l of water with gentle heating, neutralise to pH 7 $\pm$ 0.5 with 10% potassium hydroxide solution and filter if necessary. Add concentrated hydrochloric acid, to pH 1.5 $\pm$ 0.1, while still warm. Add 20 ml of salicylaldehyde and stir vigorously while heating gently (not exceeding 40 °C) for 1 h. Allow the azomethine-H to settle for 16 h. Centrifuge and discard the supernatant liquid, slurry the residue with ethanol and filter through a Whatman grade C glass-fibre filter-paper. Dry at 105 °C for 3 h and store in a desiccator. The yield of azomethine-H should be approximately 18 g.

Buffer solution. Dilute 500 ml of glacial acetic acid to 750 ml with water. Add 10.0 g of EDTA and 300 g of ammonium acetate and warm to dissolve. Adjust to pH 5.2 ± 0.1 with acetic acid or ammonia solution if necessary. Add 1.0 ml of Brij concentrate.

Azomethine reagent. Dissolve 900 ± 10 mg of azomethine-H and 2 g of ascorbic acid in 70 ml of water while heating gently (not exceeding 70 °C), and dilute to 100 ml. Add 100 ml of buffer solution and mix thoroughly. The reagent is stable for 2 d only. The solution without buffer will keep for 14 d provided that it is filtered prior to use.

Background correction reagent. Dilute 100 ml of buffer solution with an equal volume of water.

Standard solutions. A stock working standard solution of 80 mg l^{-1} of boron and an independent standard stock solution are prepared as follows. Dissolve 0.458 g of boric acid in water and dilute to 1 l in a calibrated flask. Store in a polythene bottle. Prepare the independent standard stock solution in an identical manner.

Prepare a 4.0 mg l^{-1} of boron working standard solution by diluting 5.0 ml of the stock working standard solution to 100 ml with water.

Prepare an independent standard solution containing 4.0 mg l^{-1} of boron similarly by diluting the independent standard stock solution.

Procedure

Adjust the "standard calibration" to the usual setting for 80% full-scale deflection of 4.0 mg l^{-1} (or as required). Adjust the reference filter aperture to fully open and then close down three turns. Set the base-line control nearly fully clockwise. Pump azomethine reagent through the system for 15 min and establish a flat base line by use of the sample filter aperture control with final adjustment by the base-line control.

Set 4.0 mg l^{-1} to 80% full-scale deflection with the standard calibration control. An independent standard of 4.0 mg l^{-1} should be included in the run of samples to allow for quality control. Following completion of the sample run, pump background correction reagent instead of the azomethine reagent. After 15 min, adjust the base line by opening the reference filter aperture and, if necessary, by adjusting the base-line control. Do not adjust the standard calibration or sample aperture control. Re-run the samples. The background is subtracted from the gross figure to give the actual boron concentration.

Air blips may occur at the beginning and end of each peak due to increased reagent concentrations in the segments containing sampler-introduced air. These should not interfere with peak measurement provided that the stated sample cam is used.

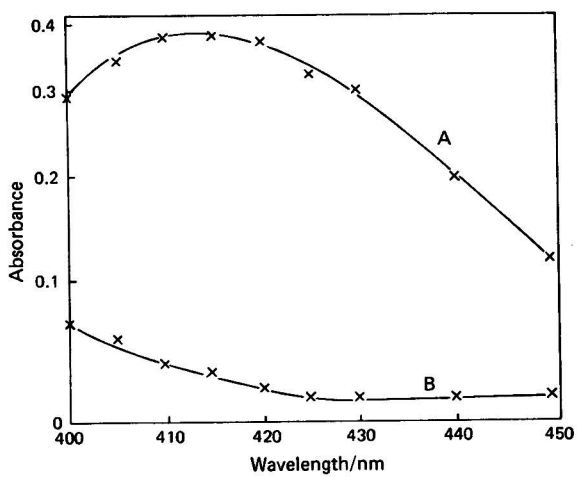

Fig. 3. Absorbance graphs for standard response (1 mg l^{-1}) relative to reagent blank. A, With boron; and B, reagent blank.

Discussion

Absorbance occurs over a wide peak at 410–420 nm, as illustrated in Fig. 3.

The effect of azomethine-H concentration on response was not tested, but Basson *et al.*[9] found that response increased with increased concentration, as illustrated in Fig. 4.

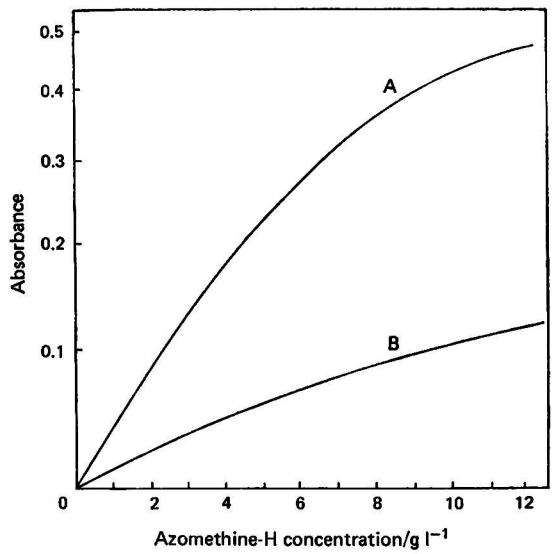

Fig. 4. Effect of azomethine-H concentration (plus L-ascorbic acid at 20 g l^{-1}) on absorbance (after Basson *et al.*). A, 5 mg l^{-1} of boron; and B, 1 mg l^{-1} of boron.

They also found that at concentrations greater than 12 g l^{-1} difficulty was experienced in obtaining a clear solution and they therefore recommended an azomethine-H concentration of 9.0 g l^{-1}.

The effect of time on colour development was tested and the results are shown in Fig. 5.

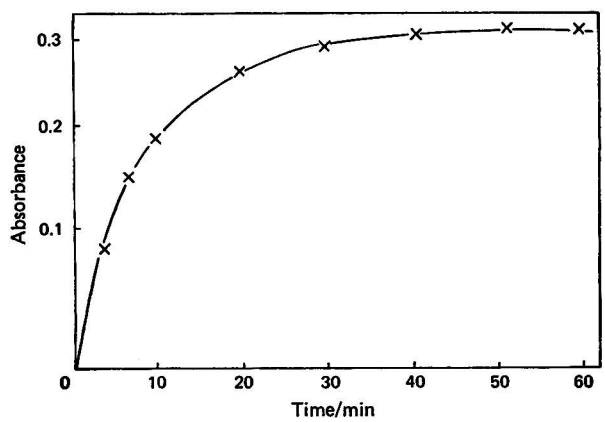

Fig. 5. Effect of time on colour development of 1 mg l^{-1} of boron (relative to reagent blank).

The time dependence graph indicated that a development time of at least 20 min was necessary for optimum response but difficulty was experienced in controlling the flow pattern with such long development times. Also, long setting-up times are incurred. A delay of 8 min was finally chosen, as this gives adequate precision and sensitivity.

With a fixed development time of 8 min the effect of development temperature was investigated. The results are illustrated in Fig. 6.

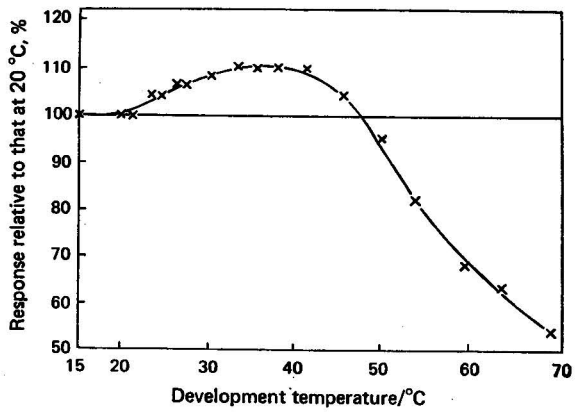

Fig. 6. Effect of development temperature on response of 1 mg l^{-1} of boron.

It can be seen that the response can be improved by over 10% by incorporating a heating bath at about 40 °C into the manifold, but this was not considered necessary for the requirements of this laboratory.

The response was found not to be linear over the whole range. Deviation from linearity was found to be significant at 4.0 mg l^{-1}, although not of practical importance, approximately 99% of linear response being obtained. However, at 5.0 mg l^{-1} a reduction to 96% of linear response was observed. All precision testing at this laboratory has assumed linearity over the range 0–4.0 mg l^{-1}.

Precision and Accuracy

Determination of boron in a range of raw waters and sewage effluents was found to be satisfactory relative to the standard carmine method,[10] which involves destruction of organic matter at 500 °C. The river waters tested gave a mean of 0.2 mg l^{-1} with a recovery of

TABLE I

RECOVERY OF BORON FROM REAL SAMPLES SPIKED AT 2.0 mg l^{-1}

Sample description*	Colour/Hazen units	Boron concentration in sample/ mg l^{-1}	Recovery, %	Significance,† 100 ± 5%
Clean river water 1	200	<0.1	106.1	N.S.
Clean river water 2	70	<0.1	103.7	N.S.
Clean peaty stream water	500	<0.1	101.5	N.S.
Good sewage effluent	40	0.89	103.3	N.S.
Poor sewage effluent	60	0.74	99.6	N.S.
Septic tank effluent	40	1.45	99.4	N.S.
Industrial maltings effluent	500	<0.1	102.1	N.S.
Detergent manufacture effluent	50	0.16	104.4	N.S.
Surface water from detergent manufacture	30	0.64	99.7	N.S.
Poor petrochemical treatment plant effluent	50	0.26	109.8	S.

* See Table II.

† N.S. = not significant at 95% confidence level; S. = significant at 95% confidence level.

TABLE II

ANALYSIS OF SAMPLES USED FOR SPIKING RECOVERY TESTS

Sample	BOD/ mg l^{-1}	Suspended solids at 105 °C/ mg l^{-1}	Ammonia (as N)/ mg l^{-1}	Total oxidised nitrogen (as N)/ mg l^{-1}	pH	Electrical conductivity/ μS cm^{-1}	Anionic detergent (Manoxol OT)/mg l^{-1}
Clean river water 1	1.5	5	0.14	0.35	6.6	90	—
Clean river water 2	0.3	1	0.41	1.8	7.2	475	—
Peaty stream water	2.1	7	0.29	0.8	6.6	100	—
Good sewage effluent	9.6	17	2.6	28.0	6.8	580	—
Poor sewage effluent	30.0	31	14.8	17.1	7.0	560	—
Septic tank effluent	—	56	17.2	0.2	7.4	800	—
Maltings effluent	234	80	1.8	0.1	7.2	1200	—
Detergent manufacture effluent	8.0	13	0.2	0.6	7.5	720	0.16
Surface water from detergent manufacture	1.2	2	0.8	17.0	7.5	3000	0.37
Petrochemical treatment plant effluent	270	84	28.8	0.1	6.5	760	—

105.3% relative to the standard method and the effluents gave a mean of 1.3 mg l^{-1} with a relative recovery of 102.9%.

Sea water gave a recovery of 100% of added boron and indicated a boron content itself of 4.7 mg l^{-1}. This compares with a calculated figure of 4.64 mg l^{-1} for a salinity of 34 parts per thousand as quoted by Barnes.[11]

Spiking recovery from real samples was tested and the results are displayed in Table I (results corrected for colour).

There was no evidence of bias in any of the effluents or raw waters tested except for the effluent containing substantial petrochemical industry waste, an effluent that would not normally be analysed for boron in this laboratory. Results of analyses of the samples used in spiking recovery tests are given in Table II.

TABLE III

SPECIES PRODUCING INTERFERENCE

Interfering species	Level producing interference/ mg l^{-1}	Interfering species	Level producing interference/ mg l^{-1}
Chromium(VI)	10	Hydrogen carbonate	1000
Iron(III)	25	Carbonate	1000
Nitrite (as N)	50	Calcium	3000
Aluminium	100	Colour	According to absorbance at 410 nm. Adequately corrected for by re-run.
Iron(II)	300		

TABLE IV

SPECIES NOT INDICATING INTERFERENCE

Species	Highest concentration tested/mg l^{-1}	Species	Highest concentration tested/mg l^{-1}
Cadmium	100	Nickel	1000
Lead(II)	100	Nitrate (as N)	1000
Sulphide	100	Orthophosphate (as P)	1000
Ammonia (as N)	1000	Silica (SiO_2)	1000
Anionic detergent (Manoxol OT, sodium lauryl sulphate)	1000	Zinc	1000
Chromium(III)	1000	Chloride	10000
Copper(II)	1000	Magnesium	10000
Fluoride	1000	Potassium	10000
Manganese	1000	Sodium	10000
Non-ionic detergent (Lissapol NX)	1000	Sulphate	10000

The method was tested for interferences by specific factors and Table III illustrates the level at which interferences became possibly significant for a recovery of boron of 100 ± 5% at a 95% confidence level. Table IV shows the highest concentrations tested for those factors that do not produce interference.

Interference by sample colour is significant and (for the river waters found within the Forth catchment) linearly related (Fig. 7); 100 Hazen units gave a background reading of 0.16 mg l^{-1} of boron. Most river waters and effluents however give a background reading below the limit of detection.

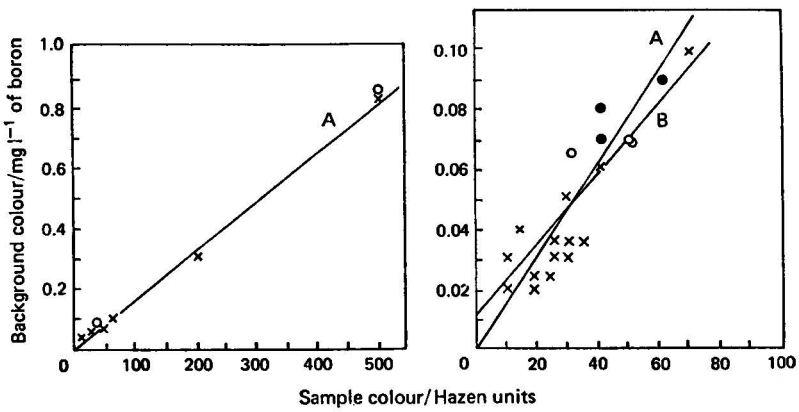

Fig. 7. Correlation between background absorbance at 410 nm and sample colour. A, Best fit for $x \leqslant 500$ Hazen units with correlation coefficient 0.999 for the whole range and 0.930 for $x \leqslant 70$ Hazen units; and B on the expanded diagram, on the right, is best fit for $x \leqslant 70$ Hazen units. ×, River water; ●, sewage effluents; and ○, industrial effluents.

Sample pH did not cause interference between pH 2 and 13.

The performance characteristics of the method were determined for standard solutions, river waters and sewage works effluents and the results are illustrated in Table V. Freshly prepared standards were analysed in triplicate on each of ten days. River water and sewage works effluents were obtained from different localities on each of ten days and analysed in triplicate. Data concerning the analysis of raw waters and effluents used are given in Table VI.

Similar analysis of blank solutions indicated a limit of detection capability (defined as $4.65S_B$, where S_B is the within-batch standard deviation of a single blank determination)

TABLE V

PERFORMANCE CHARACTERISTICS

All figures in mg l^{-1} of boron.

Parameter	Sample description*					
	0.5 mg l^{-1} standard	4.0 mg l^{-1} standard	Clean river water	Dirty river water	Good sewage effluent	Poor sewage effluent
Within-batch standard deviation	0.0170	0.0267	0.0148	0.0150	0.0156	0.0158
Between-batch standard deviation	N.S.†	N.S.	—	—	—	—
Total standard deviation ..	0.0220	0.0382	—	—	—	—
Mean	0.533	4.010	0.101	0.148	0.871	0.631

* See Table VI.

† N.S. = not significant.

TABLE VI

ANALYSIS OF SAMPLES USED FOR PRECISION TESTING

Sample type	BOD/ mg l^{-1}	Suspended solids at 105 °C/ mg l^{-1}	Ammonia (as N)/ mg l^{-1}	Total oxidised nitrogen (as N)/ mg l^{-1}	pH	Electrical conductivity/ μS cm^{-1}
Clean river water—						
Mean	1.9	9.6	0.30	3.15	7.5	495
Range	0.8–2.5	2–29	0.06–0.95	1.3–6.4	6.6–7.9	100–1070
Dirty river water—						
Mean	12.8	39.4	2.06	2.27	7.4	603
Range	5.4–24.0	11–82	0.03–5.0	1.4–3.1	6.9–8.4	440–960
Good sewage effluent—						
Mean	20.9*	22.9	13.2	6.47	7.2	639
Range	2.5–120	5–52	0.70–21.0	0.8–23.2	6.9–7.3	300–900
Poor sewage effluent—						
Mean	207	223	21.3	1.56†	7.0	856
Range	78–435	32–910	12.6–31.4	0.1–14.0	5.9–7.4	650–1020

* Elimination of one outlying BOD result gave a mean of 10.7 with a range of 2.5–36.0.
† Elimination of one outlying total oxidised nitrogen gave a mean of 0.18 with a range of 0.1–0.6.

of 0.10 mg l^{-1} and this is also indicated by the analysis of samples. Results may be defined to $\pm$0.10 mg l^{-1} or $\pm$10%, whichever is the greater (at a 95% confidence level).

Conclusion

The automated method employing azomethine-H is capable of a limit of detection of 0.1 mg l^{-1} with a precision of $\pm$0.1 mg l^{-1} or $\pm$10%, whichever is the greater. The response is virtually linear up to a level of 4.0 mg l^{-1} but decreases to about 96% at 5.0 mg l^{-1}. No important interferences have been identified except sample colour, for which adequate correction is provided. Spiking recovery from a typical range of raw waters and sanitary and industrial effluents was adequate.

The permission of the Forth River Purification Board to publish this paper is gratefully acknowledged.

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Received *July 9th*, 1979
Accepted *July 27th*, 1979

Analyst, February, 1980, Vol. 105, pp. 147–153

Divalent (Water Hardness) Ion-selective Electrodes Based on Poly(vinyl Chloride) and Poly(methyl Acrylate) Matrix Membranes

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Poly(methyl acrylate) (PMA) provides an alternative support matrix to poly(vinyl chloride) but the usual 0.17 g of polymer established for functional poly(vinyl chloride) ion-selective electrodes must be increased to 0.3 g to produce master membranes of sufficient mechanical strength. The best membrane compositions were: PMA (0.3 g), calcium bis(didecylphosphate) (0.02 g), didecylphosphoric acid (0.02 g) with either 0.09 or 0.18 g of decan-1-ol. Electrodes with such sensor membranes showed near-Nerstian response to calcium and magnesium and functioned for about 10 days. Selectivity coefficients for divalent character with respect to calcium and magnesium measured by separate and mixed solution techniques were close to unity in many instances. The useful pH range lay between approximately 2.7 and approximately 7.2 in solutions of either 10^{-1} M calcium chloride or 10^{-1} M magnesium chloride levels and at their 10^{-3} M levels.

Neither the PMA electrode nor two others based on PVC matrix membranes with divalent sensor systems permitted accurate assays of total calcium and magnesium in standard analates. However, a PVC calcium ion-selective electrode based on calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]-phosphate} and dioctyl phenylphosphonate facilitated the accurate assay of calcium in the presence of three times its amount of magnesium.

Keywords: *Ion-selective electrode with poly(methyl acrylate) matrix membrane; divalent ion-selective electrode; water hardness electrode; polymeric membranes; alkyl phosphate sensors*

Solvent mediators can exert a considerable influence on the selectivity of calcium ion-selective electrodes. Thus, calcium salts of dialkylphosphoric acids, in conjunction with dioctyl phenylphosphonate solvent mediator, produce calcium ion-selective electrodes,[1] but with decan-1-ol solvent mediator a "water hardness" (alternatively called calcium - magnesium or divalent) ion-selective electrode is produced.[2] The loss in selectivity has been used to advantage in applications of divalent electrodes for studying various aspects of water-hardness control, oceanography, biochemistry and potentiometry,[3] especially for measurements involving magnesium with calcium being present. For example, as there is no good magnesium ion-selective electrode, an indirect method for magnesium assay is to carry out a total calcium - magnesium assay with a water-hardness electrode and calcium alone with a calcium ion-selective electrode.[3]

The original design[1] of the above ion-selective electrodes, as well as those based on liquid ion exchangers and neutral carrier complexes in general, has been greatly simplified by incorporating the sensor materials in thin, flexible poly(vinyl chloride) (PVC) matrices, usually with improvement in operational lifetimes. Thus, successful calcium and water hardness ion-selective electrodes have been fabricated using a variety of sensor and mediator materials in PVC.[3] Except for poly(vinyl isobutyl ether),[4] poly(methyl methacrylate)[5] and poly(vinyl chloride/alcohol) copolymers,[6] other matrices investigated, for example, cellulose acetate,[7,8] ethylcellulose[7] and collodion,[7] are unsuitable for calcium ion-selective electrodes.

This paper describes the evaluation of one calcium and three different divalent ion-selective electrodes using a range of sensor - solvent mediator compositions in PVC and poly(methyl acrylate) (PMA) matrix membranes.

Experimental

Electrodes

Ion-selective electrodes with membranes containing the liquid ion exchanger (solvent mediator plus sensor) trapped in PVC or PMA matrices were fabricated as previously described,[9,10] except that cyanoacrylate was used to cement the PMA sensor membranes to the PVC tubing of the electrode body. The compositions of the PMA master membranes are listed in Table I. Each electrode was pre-conditioned by soaking in 10^{-1} M calcium or magnesium chloride solution for at least 24 h.

Reagents

All chemicals were of analytical-reagent grade except dioctyl phenylphosphonate, calcium bis(didecylphosphate) and calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate}, which were synthesised.[11–13] Calcium bis[di(2-ethylhexyl)phosphate] was a gift from Corning Inc., USA, and PVC [Breon S125(11)] a gift from BP Chemicals International Ltd.

Poly(methyl acrylate) was prepared by shaking methyl acrylate (100 cm^3) and azobisisobutyronitrile (0.1 g) to give a clear solution and then keeping at approximately 70 °C until a viscous monomer - polymer syrup was obtained. After dilution with toluene (100 cm^3) the mixture was slowly poured into stirred methanol (1 dm^3). The precipitated polymer was filtered, washed with methanol (1 dm^3) and vacuum dried at 35 °C. Elemental analysis: carbon 55.81%, found 55.66%; hydrogen 7.0%, found 7.1%.

Procedures

All e.m.f. measurements were made at 25 ± 0.1 °C and were relative to a Corning ceramic-junction calomel electrode (Catalogue No. 476109) with a Radiometer, Model PHM64, millivoltmeter coupled with a Servoscribe, Model RE4541, potentiometric recorder.

Selectivity coefficients were determined by separate- and mixed-solution methods.[14,15]

Results

Table I summarises the main electrochemical features of the various membranes, of which membranes VIII and IX were selected for further study and ion-selective electrode appraisal (Tables II and III, and Figs. 1 and 2). Table IV gives a comparison of the behaviour, in an analysis by standard additions, of an electrode constructed using membrane VIII with

TABLE I

COMPOSITION AND SOME ELECTROCHEMICAL PARAMETERS OF PMA MASTER MEMBRANES

Membrane No.	Master membrane composition/g: PMA	Sensor*	Decan-1-ol	Detection limit/ M × 10^{-6}	Internal reference solution	Remarks
I	0.17	—	—	—	—	Transparent, yellow membrane, but sticky on the glass casting plate
II	0.17	0.02+0.02	—	—	—	Membrane sticky on the glass plate with precipitated sensor
III	0.3	—	—	—	—	Transparent, yellow membrane, but sticky on the glass casting plate
IV	0.3	0.02+0.02	—	—	—	Membrane sticky on the glass plate with precipitated sensor
V	0.17	0.02+0.02	0.18	—	10^{-1} M $CaCl_2$	White, opaque, semi-rigid membrane, considerable surface exudate
VI	0.17	0.02+0.02	0.09	—		White, opaque, semi-rigid membrane
VII	0.3	0.02+0.02	0.36	—		White, opaque, with extensive crystalline inclusions and surface exudate
VIII	0.3	0.02+0.02	0.18	3.6	10^{-1} M $CaCl_2$	White, opaque, semi-rigid membranes. Useful pH range ~2.7–~7.2 at either 10^{-1} M $CaCl_2$ or 10^{-1} M $MgCl_2$ and similarly at either 10^{-2} M $CaCl_2$ or 10^{-2} M $MgCl_2$ levels. No pH dips below ~pH 5 as observed with all calcium ion-selective electrodes based on organophosphate sensors with dioctyl phenylphosphonate solvent mediator
				2.6	10^{-1} M $MgCl_2$	
				3.8	5×10^{-2} M $CaCl_2$, 5×10^{-2} M $MgCl_2$	
IX	0.3	0.02+0.02	0.09	3.6	10^{-1} M $CaCl_2$	White, opaque, semi-rigid membranes. pH interference profile is similar to membrane VIII
				3.6	10^{-1} M $MgCl_2$	
				3.7	5×10^{-2} M $CaCl_2$, 5×10^{-2} M $MgCl_2$	
X	0.3	0.02+0.02	0.045	1.8	10^{-1} M $CaCl_2$	White, opaque, semi-rigid membranes
				2.0	10^{-1} M $MgCl_2$	

* Calcium bis(didecylphosphate) (0.02 g) + didecylphosphoric acid (0.02 g).

that for PVC matrix-membrane electrodes with trapped calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate} plus dioctyl phenylphosphonate or decan-1-ol and with respect to a PVC matrix-membrane electrode with trapped Orion 92–32–02 liquid ion exchanger.

TABLE II

SELECTIVITY COEFFICIENTS OF PMA DIVALENT ION-SELECTIVE ELECTRODES MEASURED BY SEPARATE-SOLUTION METHODS

Membrane No.	Internal reference solution	Selectivity coefficient, $k^{Pot}_{Ca,Mg}$: At equal activity, equation (3)	Selectivity coefficient, $k^{Pot}_{Ca,Mg}$: At equal potential, equation (2)
IX	10^{-1} M $CaCl_2$	0.58–0.79	0.63–0.71
	10^{-1} M $MgCl_2$	0.78–0.82	0.74–0.79
VIII	10^{-1} M $CaCl_2$	0.60–0.76	0.63–0.67
	10^{-1} M $MgCl_2$	0.26–0.42	0.26–0.33
	5×10^{-2} M $CaCl_2$	0.76–0.86	0.77–0.83
	5×10^{-2} M $MgCl_2$		

Discussion

PMA Matrix-membrane Quality

The compatibility of any polymer matrix with solvent mediator(s) is the prime parameter affecting their ion sensor character. Thus, PVC produces soft, rubbery membranes with a wide variety of plasticising solvents, while decan-1-ol, which is a non-plasticiser, frequently gives a rigid membrane with extensive crystalline inclusions and surface exudation.[16] This type of membrane with decan-1-ol and added calcium bis(didecylphosphate) sensor proved unsuitable as a calcium-ion sensor but a functional electrode was made[16] on substituting calcium bis[di(2-ethylhexyl)phosphate]. This difference may be due to the reported plasticising character of the 2-ethylhexyl phosphate. This is supported by the fact that an electrode membrane consisting of PVC and calcium bis[di(2-ethylhexyl)phosphate], but no solvent, could be calibrated, albeit over a narrow linear range (approximately 10^{-4} to approximately 10^{-2} M), and with a slope of approximately 23 mV per decade.

PVC membranes incorporating Orion 92–32–02 divalent liquid ion exchanger also show some surface exudation. Nonetheless, ion-selective electrodes with divalent response, based on $k^{Pot}_{Ca,Mg} = 1$, can be fabricated from such membranes.[16,17] This is due, in part, to the fact that as shown[18] by thin-layer chromatography some dioctyl phenylphosphonate is present, together with decan-1-ol, among the components in the Orion 92–32–02 exchanger and also didecyl phosphate (or similar component). The dioctyl phenylphosphonate will confer a degree of plasticisation on PVC.

TABLE III

SELECTIVITY COEFFICIENTS OF PMA DIVALENT ION-SELECTIVE ELECTRODES MEASURED BY MIXED-SOLUTION METHOD

Membrane No.	Internal reference solution	Selectivity coefficient	Molarity of fixed level of interferent: 10^{-4}	10^{-3}	10^{-2}
IX	10^{-1} M $CaCl_2$	$k^{Pot}_{Ca,Mg}$	0.52	0.43	0.36
	10^{-1} M $MgCl_2$	$k^{Pot}_{Mg,Ca}$	0.83	0.91	—
VIII	10^{-1} M $CaCl_2$	$k^{Pot}_{Ca,Mg}$	0.72	0.71	0.46
	10^{-1} M $MgCl_2$	$k^{Pot}_{Mg,Ca}$	1.09	1.12	0.72

In order to obtain a divalent ion-selective electrode, it appears that incorporation of decan-1-ol is a pre-requisite for membrane fabrication by the prescribed method (Table I). Further, electrodes made from membranes V or VI containing 0.17 g of PMA were unsuitable, owing to their mechanical weakness. Thus, the amount of PMA in the master membrane was raised to 0.3 g (membranes VII–X) and adding decan-1-ol (0.18 g) and calcium bis(didecylphosphate) plus didecylphosphoric acid (0.04 g; 1:1 *m/m*) gave a good functional membrane (VIII). The resultant ion-selective electrode from membrane VIII had a slope of 29 mV per decade and a detection limit of 3.6×10^{-6} M. No real difference was evident on halving the amount of decan-1-ol (membrane IX), regarding response in pure calcium or magnesium chloride standards. Further reduction in the decan-1-ol content to 0.045 g gave a membrane (X) with properties like those of VIII and IX but with shorter lifetimes and poorer selectivities. Membrane VII, with the highest decan-1-ol content (0.36 g), showed extensive surface exudation and functional electrodes could not be fabricated.

The viable PMA ion-selective electrodes (from membranes VIII and IX) were calibrated using three different internal solutions (Table I) after pre-conditioning by soaking in 10^{-1} M calcium chloride solution for 24 h. In general, there were no clear differences in the evaluation of the electrodes with these different internal reference systems, or on calibration in pure calcium or magnesium chloride solutions. The calibration graphs for electrodes with membranes VIII or IX were near-Nerstian and the electrode operational lifetimes were about 10 d, after which response times increased and the calibration slopes fell below 28–29 mV per decade.

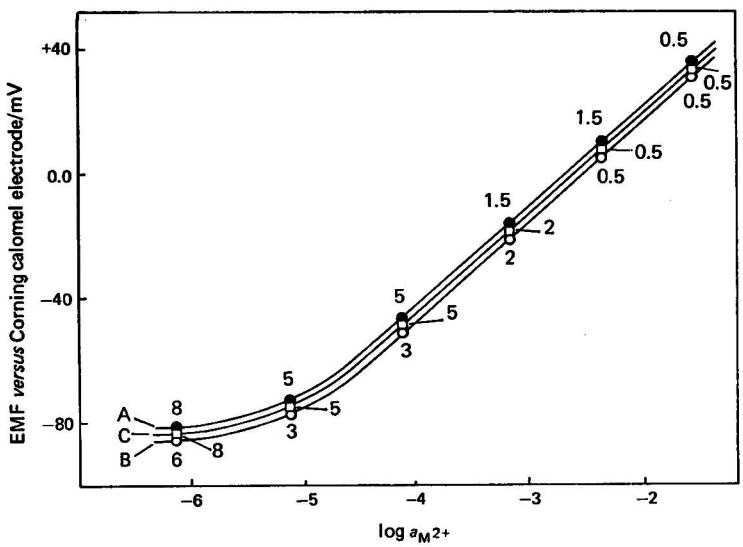

Fig. 1. Calibration graphs for PMA electrode based on membrane VIII in calcium chloride and magnesium chloride solutions. (Internal reference solution 10^{-1} M $CaCl_2$; numbers adjacent to graphs are static response times in minutes for transfer from a solution of lower to higher activity.) A, First $CaCl_2$ calibration; B, $MgCl_2$ calibration; and C, second $CaCl_2$ calibration. Slope = 29 mV per decade.

Selectivity Studies

The measurement of water hardness, defined as the sum of calcium plus magnesium in an analate, critically depends on the selectivity-coefficient parameter. Ideally, this should be unity on the basis of equation (1):

$$E = \text{constant} + S\log[a_{Ca} + k^{Pot}_{Ca,Mg} a_{Mg}] \quad .. \quad .. \quad .. \quad (1)$$

where S is the calibration slops (millivolts per decade) and a_{Ca} and a_{Mg} the appropriate cation activities.

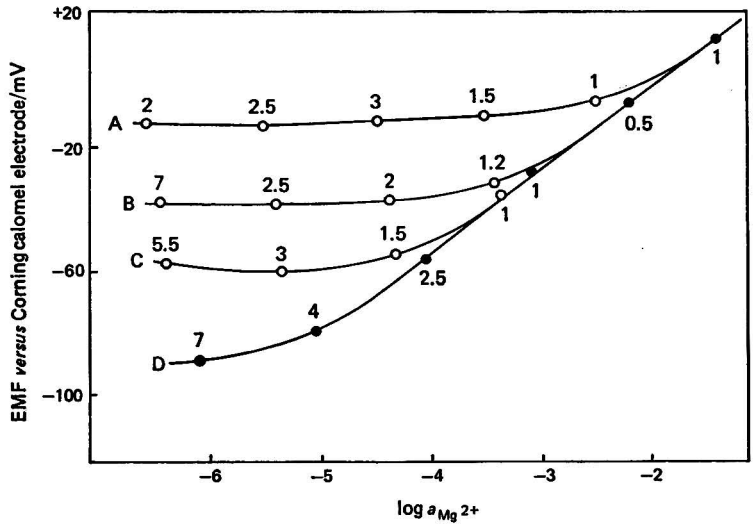

Fig. 2. Calibration, with respect to magnesium chloride, of PMA electrode based on membrane IX at three fixed levels of calcium chloride. (Internal reference solution 10^{-1} M $MgCl_2$; numbers adjacent to graphs are static response times in minutes for transfer from a solution of lower to higher activity.) A, 10^{-2} M $CaCl_2$ solution; B, 10^{-3} M $CaCl_2$ solution; C, 10^{-4} M $CaCl_2$ solution; and D, $MgCl_2$ solution only. Slope = 28 mV per decade.

In the separate-solution procedure of selectivity assessment each ion-selective reference electrode pair was first calibrated in calcium chloride standards starting with the most dilute (10^{-6} M) followed by similar successive calibrations with magnesium chloride (Fig. 1). The selectivity coefficients, $k^{Pot}_{Ca,Mg}$, were calculated first at "equal activity" using equation (2):

$$\log k^{Pot}_{Ca,Mg} = \frac{(E_{Mg} - E_{Ca})}{S} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where E_{Ca} and E_{Mg} is any pair of respective potentials taken when $a_{Ca} = a_{Mg}$.

Selectivities were also evaluated from the same graphs (Fig. 1) using equation (3) when at "equal potentials."

$$k^{Pot}_{Ca,Mg} = \frac{a_{Ca}}{a_{Mg}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

The results for three different internal reference solutions and electrodes with two different membrane compositions are shown in Table II. This separate solution procedure has been criticised on the grounds that an ion-selective electrode is used to sense an (interferent) ion for which it was not designed. However, the separate solution method is occasionally most useful, *e.g.*, for $k^{Pot}_{Ca,Zn}$, as the two e.m.f. activity calibration graphs necessary for the mixed solution technique frequently fail to coincide. Moreover, the separate-solution method permits selectivity calculation over a considerable range of activities for any one pair of primary interferent ion graphs, whereas the mixed-solution method gives just one selectivity coefficient per graph. Thus, Fig. 1 alone gives essentially a constant value for $k^{Pot}_{Ca,Mg}$ but is valid over approximately 10^{-5} to 3×10^{-2} M activity range, as the slopes of the respective calcium and magnesium calibrations are identical. This point is fundamental to the separate-solution approach, owing to the assumption of equal slopes made in deriving equations (2) and (3). However, the selectivity values never exceeded unity and indicate a small preference for calcium (Table II).

The static response times for any one electrode pair immersed in calcium or magnesium standards of the same activity was generally less than 5 min, while the corresponding potentials differed by only a few millivolts. In instances involving serious interference, potential differences are, of course, larger and as mentioned by Reinsfelder and Schultz[19] the static response times following electrode transfer from primary to interferent standards can be very long.

TABLE IV

ANALYSIS OF STANDARD CALCIUM - MAGNESIUM MIXTURES BY STANDARD-ADDITIONS METHOD USING FOUR DIFFERENT ION-SELECTIVE ELECTRODES

The results are for [calcium] and/or [calcium + magnesium] found on spiking with either calcium or magnesium as shown (0.2 cm³ and 0.1 M) using electrodes fabricated from master membranes of compositions shown, and are the mean of 5 runs with standard deviation for the run in parentheses.

Analate system (20 cm³)		Ca *iso*-OPP (0.04 g), DOPP (0.36 g) and PVC (0.17 g)*:	Ca *iso*-OPP (0.04 g), decan-1-ol (0.36 g) and PVC (0.17 g)*		Orion 92–32–02 divalent exchanger (0.4 g) and PVC (0.17 g)		Electrode made from membrane VIII	
[Ca]/ mM	[Mg]/ mM	Ca spike	Ca spike	Mg spike	Ca spike	Mg spike	Ca spike	Mg spike
10.0	—	10.6 (0.8)	12.0 (1.2)	10.7 (1.1)	11.7 (1.1)	16.4 (2.9)	11.6 (2.1)	11.8 (0.9)
7.5	2.5	7.87 (0.43)	11.7 (1.8)	11.1 (1.1)	12.5 (0.6)	16.1 (3.2)	11.9 (1.2)	13.9 (2.0)
5.0	5.0	5.33 (0.12)	13.8 (2.3)	11.3 (0.9)	12.2 (1.3)	14.2 (0.9)	8.32 (1.04)	17.6 (2.1)
2.5	7.5	2.53 (0.03)	14.6 (3.4)	11.7 (0.9)	10.6 (2.2)	12.1 (1.5)	6.23 (0.2)	15.3 (1.6)
—	10.0	0.03 (0.006)	12.9 (1.5)	12.0 (1.9)	11.0 (1.3)	12.6 (1.8)	3.63 (0.11)	12.1 (1.0)

* Ca *iso*-OPP=calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate}; DOPP = dioctyl phenylphosphonate.

Selectivity coefficients by the mixed-solution method were measured using solutions containing fixed magnesium and variable calcium activity levels (Fig. 2 and Table III) and also at fixed calcium and variable magnesium levels (Table III).

Neither class of selectivity results provides concrete evidence for real PMA divalent ion-selective character. Hence, to test this prospect further a PMA electrode based on membrane VIII was used to determine calcium or magnesium or total calcium and magnesium in standard analates using the standard-additions technique. Two other prospective divalent models, similarly employed, were based on PVC membranes with either Orion 92–32–02 exchanger (for which $k^{Pot}_{Ca\ Mg}$ is 1.0[16,17]) and calcium bis{di[4-(1,1,3,3-tetramethylbutyl)-phenyl]phosphate} plus decan-1-ol. With few exceptions the total(s) of calcium and magnesium considerably exceeded analate expectations for all three divalent electrode models (Table IV). On the other hand, a PVC calcium ion-selective electrode based on calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate} and dioctyl phenylphosphonate facilitated calcium assay at reasonably high magnesium levels. Indeed, the low value for calcium (3×10^{-5} M) in the analate containing only magnesium chloride clearly endorses the selectivity coefficient value, $k^{Pot}_{Ca\ Mg} \approx 10^{-2}$, found[20] by the mixed-solution method at $[Mg^{2+}] = 5 \times 10^{-4}$ M for this electrode.

The authors are indebted to Dr. J. Lewis for valuable assistance with the synthesis of poly(methyl acrylate).

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Received *July 25th*, 1979
Accepted *September 24th*, 1979

Biamperometric Determination of Ethanol, Lactate and Glycerol Using Immobilised Enzymes in Flow Streams

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Alcohol dehydrogenase was successfully immobilised on a nylon tube and on silanised glass beads packed in a short column, by means of covalent bonding. Lactic dehydrogenase and glycerol dehydrogenase were immobilised on titanium dioxide beads by adsorption, and on silanised glass beads by covalent bonding. All immobilisations by covalent bonding were achieved by means of glutaraldehyde cross-linking. These enzyme reactors were used in a flow system to measure ethanol, lactate and glycerol by catalysing their reaction with NAD. The NADH produced was coupled with hexacyanoferrate(III) ion, using diaphorase as a catalyst. The hexacyanoferrate(II) ion produced was measured biamperometrically using two open-tubular carbon electrodes. Measurement of standards in serum control samples was demonstrated.

Keywords: *Immobilised enzymes*; *NADH*; *hexacyanoferrate(II)*; *biamperometry*; *serum*

Alcohol dehydrogenase (ADH), lactic dehydrogenase (LDH) and glycerol dehydrogenase (GDH) catalyse the following reactions[1]:

$$CH_3CH_2OH + NAD^+ \overset{ADH}{\rightleftharpoons} CH_3CHO + NADH + H^+ \quad .. \quad .. \quad (1)$$

$$CH_3CH(OH)COOH + NAD^+ \overset{LDH}{\rightleftharpoons} CH_3COCOOH + NADH + H^+ \quad .. \quad (2)$$

$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + NAD^+ \overset{GDH}{\rightleftharpoons} \begin{array}{l} CH_2OH \\ | \\ CO \\ | \\ CH_2OH \end{array} + NADH + H^+ \quad .. \quad .. \quad .. \quad (3)$$

These reactions are often used to determine the substrate concentrations by measuring the NADH produced.[1,2] The concentration of NADH is proportional to the substrate concentration if other reactants are in high concentrations to keep the reaction pseudo-first order in the substrate.[3]

Many techniques have been used to monitor the concentration of NADH, including photometric,[1,2] fluorimetric[2] and electrochemical methods.[4–6] Amperometric determination of NADH using an oxygen electrode to measure the rate of oxygen depletion upon reaction with NADH has been employed,[7] as has amperometric measurement of hexacyanoferrate(II) ion produced by the oxidation of NADH by hexacyanoferrate(III) ion catalysed by diaphorase.[6,8] The latter reaction has been used in our laboratory for the determination of ethanol,[9] lactate[10] and glycerol,[11] using soluble enzymes and open-tubular carbon electrodes for biamperometric measurement in a flowing stream.

Immobilisation of the enzymes (the physical localisation of the enzyme molecules in a water-insoluble matrix[12]) has several advantages over the use of soluble enzymes. These include longer lifetime and less dependence on temperature increase[13] or aerobic oxidation, especially at the water - air interface.[14] The 1971 Enzyme Engineering Conference classified immobilisation methods into four categories[15,16]: (*i*) matrix entrapment, in which the enzyme molecules are entrapped within a cross-linked water-insoluble polymer such as polyacrylamide[13]; (*ii*) micro-encapsulation, in which the enzyme solution is confined in small capsules with a polymer membrane formed by dispersing the enzyme solution in an organic solution of the polymer[15]; (*iii*) immobilisation by adsorption[17]; and (*iv*) covalent bonding, by attachment of the enzyme molecule to an activated matrix with a group in the enzyme

that does not contribute to the catalytic function,[18] either by direct attachment to a group on the activated matrix,[18] or by cross-linkage through a bifunctional reagent such as glutaraldehyde.[19] The preparation of the activated matrix is achieved by liberation of an active group, *e.g.*, partial hydrolysis of a peptide linkage in nylon to liberate the primary amine group[19] or by introducing an active group into the matrix, as in silanisation of glass beads.[20,21]

A large number of adsorbants are available for the adsorption technique,[22] and the adsorbed enzymes are simple to prepare and easy to recharge.[23,24]

In this study we have investigated the immobilisation of the enzymes alcohol dehydrogenase, lactic dehydrogenase and glycerol dehydrogenase on the inside of a nylon tube and on silanised glass beads (alkylamine glass) using glutaraldehyde as a bifunctional reagent, and by adsorption on silica and titanium dioxide. The last three supports were packed in the form of columns to fit a flow system. NADU, produced in the enzyme reactions, was reacted with hexacyanoferrate(III) to form hexacyanoferrate(II), which was measured biamperometrically with tubular carbon electrodes. The most suitable immobilisation procedure for each enzyme has been determined, and the measurement of substrates in serum control samples has been demonstrated.

Experimental

Reagents

Enzymes and coenzymes. Lactate dehydrogenase (beef heart) and diaphorase were obtained from ICN Pharmaceuticals. Alcohol dehydrogenase (yeast) lyophillised powder was obtained from Calbiochem. Glycerol dehydrogenase, grade 1, and NAD were obtained from Sigma.

Lithium L(+)*-lactate.* Optically pure material was obtained from ICN Pharmaceuticals.

Potassium hexacyanoferrate(III). Reagent-grade material gave an undesirably high background, and therefore a 0.1 M solution of potassium hexacyanoferrate(III) in 0.1 M phosphate buffer at pH 7 was electrolysed at a platinum gauze electrode at +0.6 V against a saturated calomel electrode overnight. This stock solution was diluted with the appropriate buffer and the pH was adjusted as desired.

All other reagents were of ACS grade.

Methods of Immobilisation

Immobilisation by adsorption on silica and titania[25]

A 15-cm Teflon tube (3 mm i.d.) was packed with silica (42.5 nm, 0.6 $cm^3 g^{-1}$, 40 $m^2 g^{-1}$, 45–80 mesh) or titanium dioxide (65 nm, 0.3 $cm^3 g^{-1}$, 19 $m^2 g^{-1}$, 45–80 mesh), both obtained from Corning Glass Works. The tube was closed with glass-wool at both ends. Tube ending adaptors were put in both ends to fit into the flowing stream. A 0.05 M solution of phosphate buffer at pH 7.8 was pumped through this column for 2 h. Fifty units of the enzyme dissolved in 3 ml of phosphate buffer pH 7.8 were pumped through in a closed loop for 3 h. Then the column was washed with the same buffer for 30 min and stored with the buffer inside at 4 °C. Washing with buffer was carried out after each set of analyses.

Immobilisation on a nylon tube[24]

A 3-m nylon-6 tube was filled with an 18.6% *m/m* solution of water in methanol and was incubated at 50 °C for 20 min. It was then washed with 250 ml of water (2 ml min^{-1}), perfused with 3.56 M hydrochloric acid at 45 °C for 90 min (2.5 ml min^{-1}) and washed with water at 2.5 ml min^{-1} for 8 h. The tube was then perfused for 20 min with 12.5% *m/V* glutaraldehyde in 0.1 M sodium borate buffer, pH 8.5, at 2.5 ml min^{-1} and 0 °C, followed for 10 min with 50 ml of phosphate buffer, pH 7.8. Fifty units of the enzyme were dissolved in 3 ml of phosphate buffer, pH 7.8, and were pumped through the tube for 3 h with the enzyme solution inside at 4 °C and washed with 0.5 M sodium chloride in 0.1 M phosphate, pH 7.8. The tube was stored with phosphate buffer inside. Washing with pH 7.8 phosphate buffer and storage with the same buffer at 4 °C were carried out after each set of analyses.

Immobilisation on silanised glass beads[21]

To 1 g of silica beads (42.5 nm), 25 ml of a 1% *V/V* solution of 3-aminopropyltriethoxysilane in acetone were added. The mixture was evaporated to dryness and then heated at 115 °C overnight. The beads were converted into a fine powder by this process. This

powder was mixed with an equal amount of unsilanised glass beads (to enable the fluid to pass through) and packed in a 15-cm Teflon tube (3 mm i.d.). Then 25 ml of a 2.5% *m/V* solution of glutaraldehyde in phosphate buffer, pH 7, were pumped through this packed column for 90 min to activate the support inside the column, followed by exhaustive washing with distilled water. To the activated glass inside three columns, enzyme solutions (LDH, ADH and GDH), containing 50 units of enzyme in 3 ml of solution, were pumped separately (in a closed loop) for 3 h. The columns were then washed for 1 h with phosphate buffer, pH 7.5, and stored at 4 °C with buffer inside. The washing and the storing procedures were repeated after each occasion on which the column was used.

Instrumentation

The apparatus for this study is shown in Fig. 1. It consisted of a pump, sample and reagent tubes, the enzyme reactor tube or column, and the indicating electrodes and measuring circuitry.

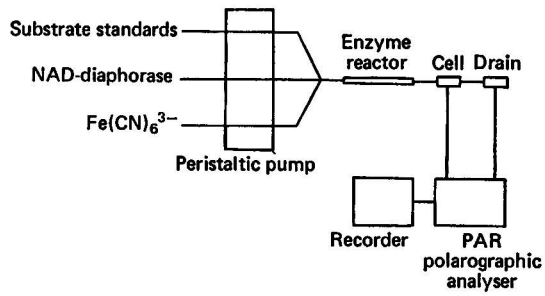

Fig. 1. Enzyme reactor - electrochemical flow system.

Tubing

All tubing, T-pieces, cross-tube connectors and tube ending adaptors were obtained from Gilson Medical Electronics. Sampling tubing for reagents and samples was of 0.03 in i.d. The sample and reagent tubes were connected in a cross-adaptor to the reactor tube or column, followed by the tubular electrodes.

Pump

A 4-channel Gilson Minipulse II peristaltic pump was used throughout.

Cell

A carbon rod from a standard 1.5-V battery approximately 8 mm in diameter was cut into two 2-cm lengths and a 0.8-mm hole was drilled down the axis of each rod. Indentations of 3 mm diameter were then drilled into the ends of the electrodes, and the Tygon tubes were glued into these openings. The electrodes were separated by a 2-cm length of tubing. The emptying end of the flow tubing was kept well downstream from the second electrode to avoid pulsations due to pressure changes in the tubes.

Voltage source

A PAR-174 polarographic analyser was used to set the voltage between the electrodes at 200 mV and to measure the current. An *X* - *Y* recorder was used to monitor the current.

Procedures

Determination of ethanol by ADH immobilised on a nylon tube

Ethanol standards in tris(hydroxymethyl)aminomethane (Tris) buffer, pH 8, were introduced in one tube. A 0.001 M solution of potassium hexacyanoferrate(III) in the same buffer and at the same pH was introduced into the second tube. A reagent solution containing 0.001 M NAD^+ and 1.5 units ml^{-1} of diaphorase, all in the same Tris buffer at pH 8,

was introduced into the third tube. These solutions were mixed at the cross in the flow stream and then entered the nylon tube with ADH immobilised inside it. The pump rate was adjusted to permit a reaction time of 6 min. Sample consumption was 75 μl min^{-1} and the sampling time was 2 min.

Determination of ethanol by ADH immobilised on silanised glass beads

Standards of ethanol in aqueous Tris buffer and in a buffered solution of serum control samples, which had been diluted 20-fold in the same buffer, all at pH 7.5, were mixed at the cross in the flow stream with a solution of 0.005 M potassium hexacyanoferrate(III) and 0.01 M NAD^+ containing 1.5 units ml^{-1} of diaphorase, all in Tris buffer at pH 7.5. The mixture was introduced into the reaction column containing the immobilised ADH. A reaction time of 4 min was obtained by adjusting the pumping rate. The sample consumption was 100 μl min^{-1} and the sampling was for 2 min. A stable signal was obtained in the second minute after the sample had reached the cell. A recovery study of ethanol added to serum control samples was performed using this method.

Determination of lactate by LDH immobilised on silica or titanium dioxide beads

Standards of lithium L(+)-lactate in aqueous Tris buffer at pH 8 were mixed at the cross-adaptor with the same reagent solutions as above for ADH immobilised on silanised glass beads. The pumping rate was adjusted to permit a reaction time of 4 min. Sampling was carried out for 2 min with a rate of sample consumption of 100 μl min^{-1}. A stable signal was obtained after 1 min for each sample passing through the cell. The immobilised LDH on titanium dioxide gave a larger signal than that immobilised on silica.

Determination of lactate by LDH immobilised on silanised glass beads

Standards of lithium L(+)-lactate in both aqueous buffer and buffered serum control samples, which had been diluted 20-fold, all in 0.1 M Tris buffer at pH 7, were mixed with the same reagents and in the same manner as above for the silica and titanium dioxide beads. The reaction time, sampling time and rate of sample consumption were also the same. A stable signal was obtained after 1 min for each sample passing through the cell.

Determination of glycerol by GDH immobilised on silica or titanium dioxide beads

The procedure for glycerol was exactly as above for LDH on silica or titanium dioxide beads, except that the buffer used was 0.05 M glycine at pH 8 and 0.1 M ammonium chloride

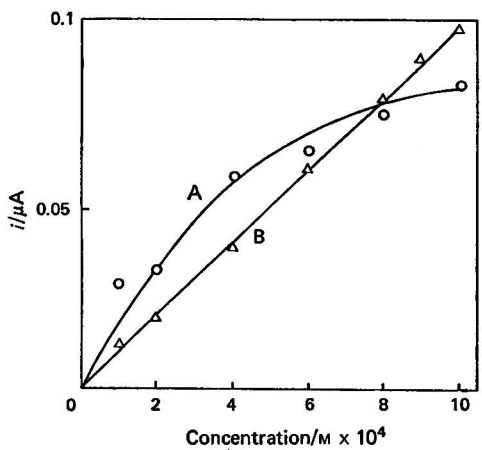

Fig. 2. Calibration graphs using enzymes immobilised on titanium dioxide. A, Determination of lactate in aqueous buffer solutions using LDH; and B, determination of glycerol in aqueous buffer solutions using GDH.

solution was added to the reagent as an activator to GDH.[11] Standards of glycerol in aqueous buffer were analysed.

Determination of glycerol by GDH immobilised on silanised glass beads

The same procedure was followed as above for GDH on silica or titanium dioxide, except that the pH was 8.5. Standards of glycerol in aqueous buffer, glycerol in buffered serum control samples diluted 20 times and standards of tristearin after hydrolysis with lipase and in the same buffer were analysed.

Blank (background) currents were determined for aqueous solutions by replacing the sample with buffer solution. For serum samples, the unspiked samples were run, and NAD was omitted from the reagent. Net current readings are reported.

Results and Discussion

Immobilisation on a Nylon Tube

This technique was not satisfactory with glycerol dehydrogenase and gave a very weak signal with lactic dehydrogenase, which was insufficient to establish a calibration graph at the proper concentrations. However, with alcohol dehydrogenase, a weak but adequate signal was obtained, sufficient to establish a slightly non-linear calibration graph in the range 5×10^{-4}–10^{-2} M ethanol.

Immobilisation by Adsorption on Silica and Titanium Dioxide Beads

This immobilisation technique was not successful with alcohol dehydrogenase but was satisfactory with lactic dehydrogenase and glycerol dehydrogenase. Both carriers gave positive results with these two enzymes but the signal using titanium dioxide was greater than that using silica. The activity of the enzymes lasted 2 months, but daily calibration was required. Fig. 2 shows the calibration graphs for the determination of lactate and glycerol in aqueous buffer solutions using LDH and GDH immobilised on titanium dioxide beads. Glycerol and lactate gave approximately the same currents, but the glycerol measurements gave much superior linearity and precision.

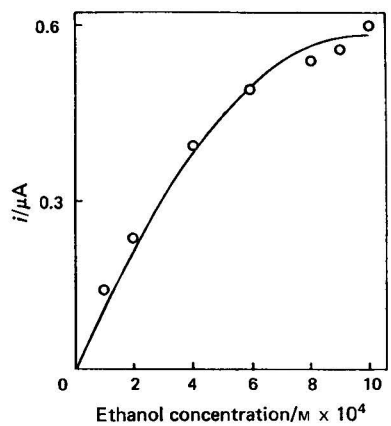

Fig. 3. Calibration graph for the determination of ethanol in serum control samples using ADH immobilised on silanised glass beads. Concentrations are those in the diluted samples.

Immobilisation by Covalent Attachment to Silanised Glass Beads

This technique of immobilisation was successful for all three enzymes. As silanised and unsilanised glass beads were mixed to enable the fluid to flow through the column, partial

TABLE I

RECOVERY OF ETHANOL ADDED TO SERUM CONTROL SAMPLES

All samples were diluted 20-fold after the addition of ethanol.

Ethanol added/ mg per 100 ml	Ethanol found*/ mg per 100 ml	Difference/ mg per 100 ml	Recovery, %
100	92	−8	92
100	95	−5	95
150	148	−2	99
200	194	−6	97
300	258	−42	86

* Mean results of three replicate determinations.

immobilisation by adsorption is expected. The activity of the enzymes immobilised by this method lasted more than 2 months without the need for daily calibration. The activity remained constant for the duration of this study.

Fig. 3 shows the calibration graph for the determination of ethanol in buffered serum control samples. The results were linear up to 4×10^{-4} M in the diluted serum. A similar graph was obtained using aqueous solutions of ethanol in buffer. Table I shows the recovery of ethanol added to serum control solutions.

Linearity was obtained up to 6×10^{-4} M for the determination of lactate, as shown in Fig. 4. The calibration graph for the determination of lactate in serum control samples is also shown in Fig. 4. The non-zero intercept is due to lactate present in the sample. The slope is reduced in the presence of serum, and pooled serum or serum control samples must be used for calibration. Alternatively, a standard-additions procedure can be used.

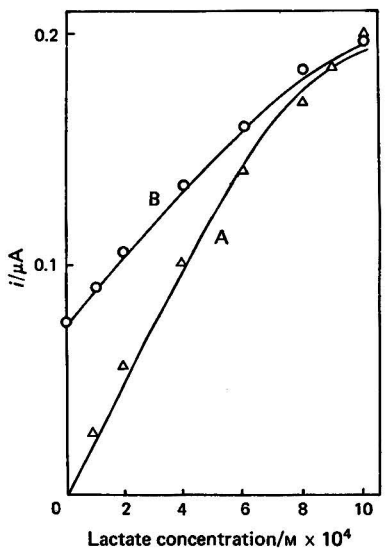

Fig. 4. Calibration graphs for the determination of lactate using LDH immobilised on silanised glass beads by covalent bonding. A, Aqueous buffer solutions; and B, serum control samples diluted 1 + 20 with buffer.

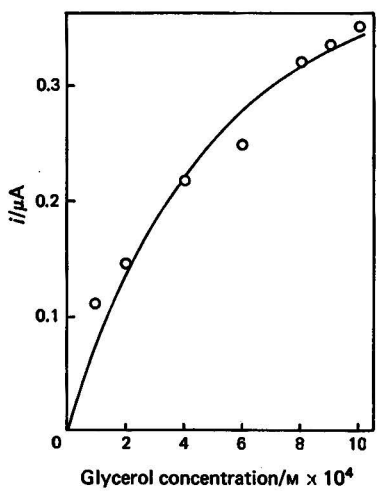

Fig. 5. Calibration graph for the determination of glycerol in aqueous buffer solutions using immobilised GDH on silanised glass beads by covalent bonding.

The calibration graph for glycerol in aqueous buffer solution is shown in Fig. 5. It is non-linear in the range 10^{-4}–10^{-3} M. Similar calibration graphs were obtained using standards of tristearin (after hydrolysis) in aqueous solution, and standards of glycerol in serum control samples, with a non-zero intercept for the latter due to the glycerol in the sample.

pH Effect

Immobilisation of enzymes frequently causes a displacement of the optimum pH.[26] The maximum activities of the native enzymes used in this study were at pH 8.5 for ADH,[9] 8.0 for LDH[10] and 8 for GDH.[11] After immobilisation, the optimum pH is shifted in each instance. The optimum pH for ADH immobilised by covalent bonding to silanised glass beads was 7.5, for LDH it was pH 7 and for GDH it was between pH 8.5 and 9.

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Received *July 16th*, 1979
Accepted *August 30th*, 1979

Analyst, February, 1980, Vol. 105, pp. 161–164

Analytical Methods Committee

REPORT PREPARED BY THE MEDICINAL ADDITIVES IN ANIMAL FEEDS SUB-COMMITTEE "A"

Determination of Ronidazole in Animal Feeds by Gas - Liquid Chromatography: A Collaborative Study by the EEC Committee of Experts

A previously published gas - liquid chromatographic procedure with flame-ionisation detection for the determination of ronidazole in feeding stuffs has been investigated by inter-laboratory studies and with minor modifications has been recommended as a method for use by the EEC. Collaborative studies have shown the method to be acceptable for enforcement purposes. The ronidazole is extracted from the feeding stuff with ethyl acetate and the solution is cleaned up by transferring the ronidazole, as its hydrochloride, into an aqueous solution, liberating the ronidazole by making the solution alkaline and re-extracting with ethyl acetate. The solvent is then evaporated, the ronidazole converted into a volatile silyl derivative by reaction with *NO*-bis(trimethylsilyl)acetamide in hot quinoline solution and the reaction mixture, with the addition of diethyl phthalate as internal standard, is then examined by gas - liquid chromatography.

Keywords: *Ronidazole determination*; *animal feeds*; *coccidiostats*; *gas - liquid chromatography*; *silylation*

The Analytical Methods Committee has received and approved for publication the following Report from its Medicinal Additives in Animal Feeds Sub-Committee "A."

Report

This Report is the result of collaborative experimental work by members of the Committee of Experts on Determination of Coccidiostats of the European Economic Community (EEC) together with members of AMC Medicinal Additives in Animal Feeds Sub-Committee "A." The Member States of the EEC participating in this study were Belgium, Denmark, Germany, Ireland, Italy, The Netherlands and the United Kingdom, under the Chairmanship of Dr. S. Dormal van den Bruel; this Report is published with their agreement.

The constitution of the AMC Sub-Committee responsible for the preparation of this Report was: Mr. J. Markland (Chairman), Mr. R. J. Anderson, Mr. A. G. Croft, Mr. R. Fawcett, Dr. K. Field (until 1978), Mr. J. R. Harris (from 1978), Mr. R. S. Hatfull, Mr G. E. Kitson, Mr. D. H. Mitchell and Mr. J. A. Stubbles, with the late Dr. N. W. Hanson as Secretary.

Introduction

Ronidazole [(1-methyl-5-nitroimidazol-2-yl)methyl carbamate] is used in poultry feeds for the control and treatment of blackhead; it is also used in pig feeds for the control and treatment of dysentery and as a growth-promoting agent. The normal level of inclusion of the drug in a feed is between 60 and 120 mg kg^{-1}.

A recommended method for the spectrophotometric determination of ronidazole in animal feeds has been published,[1] but reservations regarding interferences made it necessary for the EEC to consider a method based on gas - liquid chromatography.[2]

It was decided that this method would be subjected to collaborative study and the UK was requested to provide samples of feeds medicated with ronidazole.

Experimental and Results

Two samples, a pig feed and a poultry feed, to which ronidazole had been added at concentrations of 100 and 60 mg kg^{-1}, respectively, were circulated to participating laboratories. Samples of the unmedicated feeds were also sent to participants. The results submitted by the participating laboratories are given in Table I.

It was apparent from the comments received from participants that several points of detail required further clarification. In particular the pH of the solution in the clean-up stage, before re-extracting with ethyl acetate, should be between 11 and 12. Also, the conditions for silylation should be more closely defined: heat the reaction mixture at 150–155 °C for 10 min (preferably in a metal heating block). These points have been incorporated in the method (see Appendix).

TABLE I

DETERMINATION OF RONIDAZOLE IN MEDICATED FEEDS

	Amount of ronidazole found/mg kg^{-1}			
Laboratory	Pig feed + 100 mg kg^{-1} of ronidazole	Pig feed blank	Turkey feed + 60 mg kg^{-1} of ronidazole	Turkey feed blank
1	89.6, 86.6, 88.9, 82.8	0	48.3, 46.7, 51.8, 48.3	0
2	100, 93, 96	3.5	54, 58, 56	6.4
3	80.0, 81.0	6.4	58.9, 57.9	—
4	88, 94	0	57, 54	0
5	90.9, 92.4, 93.1	0	55.7, 54.3, 54.3, 56.0	0
6	100.3	4.9	56.1	2.5
7	95.3, 92.2	2.5	56.0, 58.0	2.0
8	97.0	0	55.0, 53.0	0
9	92.0, 83.0	<2	50.0, 55.0	<2
10	119, 114	—	64, 58	—
11	91.7, 95.7	—	66.7, 62.4	—

Statistical analysis—

	Pig feed	Turkey feed
Number of results	24	26
Mean/mg kg^{-1}	93.18	55.59
Range/mg kg^{-1}	39 (80.0–119.0)	20 (46.7–66.7)
Standard deviation/mg kg^{-1}	9.03	4.58
Coefficient of variation, %	9.69	8.24
Repeatability standard deviation/mg kg^{-1}	3.57 (based on 18 results)	2.43 (based on 20 results)
Repeatability/mg kg^{-1}	10.09	6.87

APPENDIX

Determination of Ronidazole in Animal Feeding Stuffs

1 Purpose and Scope

A gas - liquid chromatographic procedure is described for the determination of ronidazole [(1-methyl-5-nitroimidazol-2-yl)methyl carbamate] in animal feeding stuffs.

The lower limit of the determination is 25 mg kg^{-1}.

2 Principle

The ronidazole is extracted from the feeding stuff with ethyl acetate. The extract is subjected to a clean-up stage, evaporated to a small volume and the ronidazole is reacted with *NO*-bis(trimethylsilyl)acetamide (BSA) to form a volatile silyl derivative, which is examined by gas - liquid chromatography.

3 Reagents

3.1 *Sodium chloride.*
3.2 *Sodium sulphate, anhydrous.* Heat overnight at 350 °C.
3.3 *Ethyl acetate.*
3.4 *Quinoline.*
3.5 NO-*bis(trimethylsilyl)acetamide (BSA) silylating reagent.*
3.6 *Trisodium orthophosphate solution,* 5 g *per* 100 ml.
3.7 *Hydrochloric acid,* 1 M.
3.8 *Sodium hydroxide solution,* 5 M.
3.9 *Internal standard solution.* Dissolve 25.0 mg of diethyl phthalate in quinoline and dilute to 50.0 ml with quinoline (3.4).
3.10 *Ronidazole standard solution.* Weigh, to the nearest 0.1 mg, approximately 25 mg of pure ronidazole, dissolve in ethyl acetate and make the volume up to 100.0 ml with ethyl acetate. This solution is stable for 48 h.

4 Apparatus

4.1 *Rotary evaporator.*
4.2 *Gas chromatograph.* Suitable for on-column injection and fitted with a flame-ionisation detector.

The following conditions are offered for guidance.

4.3 *Column.* Glass, i.d. 4 mm and length 1.5 m.
4.3.1 *Support.* Gas-Chrom Q, mesh size 80–100 μm.
4.3.2 *Stationary phase.* 5% OV-17.
4.4 *Column temperature.* 165 °C.
4.4.1 *Injector temperature.* 220 °C.
4.4.2 *Detector temperature.* 300 °C.
4.5 *Carrier gas.* Nitrogen, flow-rate 30 ml min^{-1}.
4.5.1 *Fuel gases.* Hydrogen, flow-rate 30 ml min^{-1}, and oxygen, flow-rate 300 ml min^{-1}.
4.6 *Extractor unit.* Soxhlet type.
4.7 *Test-tube.* Capacity 15 ml, with standard ground-glass neck.
4.8 *Micro-filter.* Sintered glass, porosity 1, i.d. 2.3 cm, length 12 cm.

5 Procedure

5.1 *Extraction*

Weigh, to the nearest 0.001 g, a portion of finely divided and mixed sample containing between 450 and 600 μg of ronidazole. Grind the sample in a mortar with 2 ml of trisodium orthophosphate solution (3.6) and transfer the mixture into an extraction thimble. Extract the mixture in a Soxhlet apparatus (4.6) with 125 ml of ethyl acetate (3.3), allowing about 20 cycles, and then cool the solution. Reduce the volume of the ethyl acetate extract to about 30 ml using a rotary evaporator (4.1) at a temperature of 30 °C, and transfer the solution into a 100-ml separating funnel. Rinse the flask several times with 2-ml portions of ethyl acetate and add the washings to the funnel.

5.2 *Removal of interfering substances*

Extract the ethyl acetate solution (5.1) with three 10-ml portions of 1 M hydrochloric acid (3.7) and combine the acid extracts in a second 100-ml separating funnel. Add 5 ml of ethyl acetate (3.3) to the acid extract, swirl gently and run off the acid layer into a 250-ml separating funnel. Add 10 g of sodium chloride (3.1) and swirl the funnel to produce a saturated solution. Make the solution alkaline with 5 M sodium hydroxide solution (3.8), to pH 11–12, keeping the time of exposure of ronidazole to the sodium hydroxide solution as short as possible, and immediately extract with three 40-ml portions of ethyl acetate (3.3). Run the ethyl acetate extracts through a micro-filter (4.8), containing 20 g of sodium sulphate (3.2), into a 250-ml round-bottomed flask. Wash the filter with two 10-ml portions of ethyl acetate (3.3) and add the washings to the flask.

5.3 *Silylation*

Reduce the volume of the ethyl acetate extract (5.2) to about 3 ml in a rotary evaporator (4.1) at 30 °C. Quantitatively transfer the remaining extract into a 15-ml test-tube (4.7) and rinse the flask with four 2-ml portions of ethyl acetate (3.3). Evaporate the mixture to dryness on a water-bath at 50 °C under a stream of nitrogen and add 1.0 ml of the internal standard solution (3.9) to the residue. Add 250 μl of BSA silylating reagent (3.5) with a syringe and gently shake the tube. Fit a condenser to the tube and heat at 150–155 °C for 10 min (a metal heating block is recommended). Remove the tube from the heat source and allow it to cool to room temperature. Inject 5 μl of this solution on to the column. Measure the peak height of the ronidazole and diethyl phthalate internal standard peaks. The retention times of the ronidazole and diethyl phthalate are approximately 12 and 14 min, respectively.

5.4 *Ronidazole standard*

Transfer, by pipette, 2.0 ml of standard ronidazole solution (3.10) into a 100-ml separating funnel and make the volume up to 30 ml with ethyl acetate (3.3). Proceed as described above from *Removal of interfering substances* (5.2).

6 Calculation of Results

Calculate the peak-height ratio of the ronidazole to diethyl phthalate peaks for the feed sample and the ronidazole standard. The amount of ronidazole, in milligrams per kilogram, in the feed is given by the equation

$$\text{Ronidazole} = \frac{2 \times A \times C}{B \times M}$$

where

A = peak-height ratio derived from feed.
B = peak-height ratio derived from ronidazole standard.
C = concentration of ronidazole standard (micrograms per millilitre).
M = mass of feed sample taken (grams).

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SHORT PAPERS

Study of Certain 1,5-Benzodiazepine Derivatives as Acid - Base Indicators

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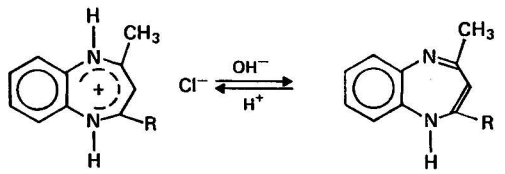
Keywords: *1,5-Benzodiazepine derivatives; acid - base indicators; dissociation constants; potentiometric measurement*

Current interest in the synthesis and investigation of the physical properties of certain aza-carbonium heterocyclic compounds can be extended to the study of the fundamental chromogenic properties of 2,4-disubstituted 1,5-benzodiazepinium salts, such as compounds **I–VII**. These compounds were prepared, essentially, as intermediates for the synthesis of 6,7-benzo-1,2,4,5-tetrahydro-1,5-diazepines as potential psychotropic agents.[1]

The deep violet colour of these conjugated cations was discharged by alkalis and restored by acids (Scheme I).



(Violet/pink) (Colourless/yellow)

R	Compound
CH_3	I
C_6H_5	II
p-C_6H_4Cl	III
p-C_6H_4Br	IV
p-$C_6H_4CH_3$	V
p-$C_6H_4OCH_3$	VI
m-$C_6H_4NO_2$	VII

Scheme 1

The use of compounds **I–VII** as pH indicators has been investigated in terms of their pK_a values and the effect of the medium on these values.

Experimental

Apparatus

A Radelkis pH meter (OP-401/2) fitted with a sealed calomel sleeve-type electrode (OP-810) and a shielded glass electrode (OP-717/1A) were used, and a Jean and Constant Prolabo spectrometer with 1-cm thick glass cells.

Reagents and Materials

All reagents were of analytical-reagent grade, and boiled and cooled doubly distilled water was used.

Sodium metal.

Potassium hydroxide, carbonate free.

Hydrochloric acid.

Benzoic acid. The accurate content of the benzoic acid was determined according to the E.P. method.

Methanol, anhydrous. Prepared as recommended in reference 2.

Titrants and solutions. Aqueous standard buffer solutions were used for calibration of the pH meter; 0.02 M sodium methoxide solution (in anhydrous methanol), aqueous 0.02 M potassium hydroxide solution, both bases, were standardised against benzoic acid in methanol. Hydrochloric acid (0.02 M) was standardised in terms of one of the prepared alkaline standards. Solutions (0.004 M) of each of the compounds to be investigated were prepared so as to obtain relatively dilute solutions at the half-neutralisation points. All titrations were performed in an atmosphere of nitrogen, with stirring.

Indicators. 2,4-Disubstituted-6,7-benzo-1,5-diazepinium chlorides (**I–VII**) were synthesised and purified through the condensation of *o*-phenylenediamine and the appropriately substituted β-diketones, as previously reported.[1] Phenolphthalein, methyl orange and the 1,5-benzodiazepine salts, as 0.1% solutions in methanol, were used for the determination of the end-points.

Methods

Determination of the dissociation constants of compounds **I–VII** was carried out according to Soliman.[3] However, the pH at the half-neutralisation points, which correspond to the apparent pK_a values, were obtained from practical titration curve data. The pK_a values determined for each of the compounds investigated in different concentrations of aqueous methanol were plotted against the concentration of methanol, and the values extrapolated to 0% of methanol to give the $pK_{a(water)}$ of the compound being studied (Fig. 1).

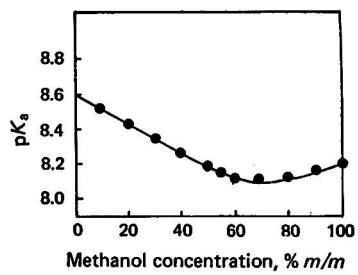


Fig. 1. Effect of methanol concentration on the dissociation process of compound **I**.

The effect of the medium of methanol and the different water - methanol mixtures was determined by the use of the following equation[4,5]:

$$pK_s = p(K_{HIn})_s - p(K_{HIn})_w = \log \frac{(f)_H + (f)_{In}}{(f)_{HIn}}$$

where s and w represent solvent and water, respectively, and f the activity coefficient of the medium.

Results and Discussion

For the preliminary evaluation of compounds **I–VII** as acid - base indicators, their methanolic solutions were used in the titration of 0.02 M hydrochloric acid and potassium hydroxide solutions. Parallel titrations were carried out using phenolphthalein and methyl orange as reference indicators. The results obtained were compared with those determined potentiometrically.

As shown by the results in Table I, compounds **I–VII** gave results comparable to those obtained using phenolphthalein and methyl orange, but they give a more distinct and sharp colour transition than the methyl orange indicator.

TABLE I

VISUAL TITRATIONS OF 0.02 M HYDROCHLORIC ACID AND POTASSIUM HYDROXIDE SOLUTION

The potentiometric determination is taken as 100%; the visual titration results are the average of five determinations relative to the potentiometric result.

Compound	Acid - base colour change	HCl found, %	Standard deviation, %	KOH found, %	Standard deviation, %	Colour in water, $\lambda_{max.}$
I	Pink—yellow	101.0	0.21	99.3	0.25	480
II	Violet—yellow	100.6	0.24	100.2	0.30	485
III	Violet—yellow	100.4	0.32	100.4	0.27	485
IV	Pink—yellow	100.2	0.25	100.5	0.33	470
V	Violet—colourless	100.4	0.25	100.3	0.32	480
VI	Violet—colourless	100.3	0.27	100.4	0.41	485
VII	Pink—pale yellow	100.1	0.30	100.3	0.42	465
Methyl orange	Orange red—yellow	98.6	0.35	102.5	0.31	—
Phenolphthalein	Colourless—pink	100.8	0.30	100.5	0.33	—

However, the relatively low solubility in water of the salts investigated (Table II) presented some difficulties in the determination of their pK_a values. This difficulty was overcome by determination of the pK_a value of each salt in solutions at different concentrations of methanol in water (Fig. 1). The method is essentially based on that used by Soliman[3] for the determination of dissociation constants of water-insoluble compounds.

TABLE II

SOLUBILITY AND MOLAR-ABSORPTIVITY DATA FOR SUBSTITUTED 1,5-BENZODIAZEPINES IN WATER AND METHANOL

Compound	R	Solubility* at 25 °C		Molar absorptivity at $\lambda_{max.}$, 1 mol^{-1} cm^{-1}	
		Water	Methanol	Water	Methanol
I†	CH_3	10	10	1.06×10^3	0.95×10^3
II†	C_6H_5	100	50	1.39×10^3	1.32×10^3
III†	*p*-C_6H_4Cl	1200	500	1.60×10^3	1.33×10^3
IV	*p*-C_6H_4Br	1200	500	1.61×10^3	1.35×10^3
V	*p*-$C_6H_4CH_3$	150	100	1.10×10^3	1.37×10^3
VI	*p*-$C_6H_4OCH_3$	100	100	1.73×10^3	1.80×10^3
VII	*m*-$C_6H_4NO_2$	2000	1000	1.52×10^3	1.71×10^3

* Solubility expressed as millilitres of the solvent that dissolve 1 g of the compound.
† Certain salts, compounds I, II and III, are moderately soluble in water but their free bases, which are released during neutralisation, are insoluble, thus hindering accurate determination of the pK_a values in water. This is not the case in methanol.

The values of $pK_{a_{(water)}}$ and $pK_{a_{(methanol-water)}}$ for compounds I–VII are given in Table III.

The values of $pK_{a_{(water)}}$ may be considered as the thermodynamic constants, as all measurements were carried out at relatively high dilutions.[6] A Hammett plot showed a good linear relationship for the salts investigated, with $\rho = -1.44$ (Fig. 2).

This observation is very important in predicting the nature of the substituent most suitable for the synthesis of the derivative that will change its colour in the required pH range.

To illustrate the effect of the medium on the apparent pK_a values the relation between ΔpK_a and the concentration of methanol is given in Fig. 3.

As shown by the data given in Table III and Fig. 3, it is evident that by changing the salts investigated from solution in water to solution in methanol there is a gradual decrease in the corresponding pK_a value, until it reaches a minimum value at a methanol concentration of about 60%. An elevation of the pK_a value is observed, however, on further increase in the methanol concentration. However, the over-all effect of the medium on the initial pK_a values is comparatively small. Such a result can be attributed to the facile transfer in the investigated compounds from aqueous to methanolic media, irrespective of the

TABLE III

DISSOCIATION CONSTANTS OF COMPOUNDS I–VII IN WATER AND IN WATER - METHANOL MIXTURES

		pK_a value						
			Water - methanol mixtures: methanol concentration, %					Hammett constant,
Compound	R	Water	20	40	60	80	100	σ
I	CH_3	8.60	8.44	8.27	8.10	8.10	8.20	—
II	C_6H_5	6.50	—	—	—	—	—	—
III	p-C_6H_4Cl	6.20	6.13	6.05	5.95	6.00	6.28	0.23
IV	p-C_6H_4Br	6.15	6.17	6.17	6.18	6.19	6.30	0.27
V	p-$C_6H_4CH_3$	6.70	6.57	6.47	6.40	6.45	6.97	−0.17
VI	p-$C_6H_4OCH_3$	6.86	6.71	6.55	6.41	6.41	7.09	−0.27
VII	m-$C_6H_4NO_2$	5.53	5.52	5.52	5.51	5.53	5.65	0.71

changes brought about in the dielectric constant. As has been established previously by Born,[7] similar transfers are characteristic of charged molecules, to which species the salts investigated can obviously be related. Because of the cationic nature of the salts and the mentioned effect of the medium, compounds I–VII can be grouped under A^+B°-type indicators.[8]

It was of interest to trace the regularity shown by substitution of different groups on the variation of the pK_a values as a result of the effect of the medium. Initially, such regularities were established by Hammett for the behaviour of charged molecules: $p(K_aR)_w - p(K_aR)_s$ = constant, where the relation is given for different substituent members (R) of the same charged derivative in water, $(K_a)_w$, and the medium being investigated, $(K_a)_s$, respectively.[9] As shown by the data given in Fig. 3, such regularities were not available for compounds II–VII.

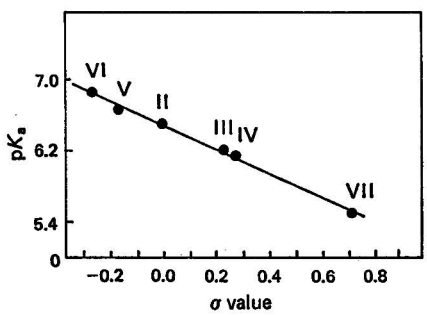


Fig. 2. Relation between pK_a and Hammett constants of compounds II–VII.

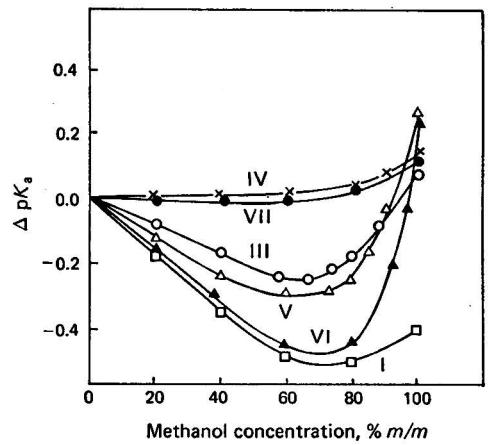


Fig. 3. Effect of methanol concentration on ΔpK_a of compounds I, III–VII, $pK_a = (pK_a)_s - (pK_a)_w$.

Similar deviations have also been reported by other workers.[10] It is possible that the substitution effect within the salts investigated upon the regularity of the pK_a values is not only influenced by the charge parameter brought about by the given substituent (R), but is affected most by other physicochemical factors, *e.g.*, van der Waals radii, electron density or even by a multiplicity of the given factors.

Conclusions

The 2,4-disubstituted 1,5-benzodiazepinium chlorides investigated have been shown to have acid - base characteristics so as to be of use as pH indicators within the pH range 5–9. Choice of the required pH interval can be affected by the nature of the substituent in the 4-position.

Because of the relatively facile synthesis of the benzodiazepinium salts, and the wide range of the 2- and 4-substituents, they offer a new class of acid - base indicators.

Violation of the validity of the Hammett acidity function [H_0] is presented by variation of the ΔpK_a values of the salts investigated. Factors other than the type of charge of the indicators should be taken into consideration for the study of the effect of the medium in this series of diazepinium salts.

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Received *July 10th*, 1979
Accepted *August 30th*, 1979

Spectrophotometric Determination of Oxyphenbutazone in Pharmaceutical Preparations

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Keywords: *Oxyphenbutazone determination*; *spectrophotometry*; *pharmaceutical preparations*

Despite the importance of oxyphenbutazone as an analgesic and anti-inflammatory drug, little has been published concerning its determination in pharmaceutical preparations. The reported methods include titrimetric[1] and spectrophotometric[2–4] methods. In this work two colour reactions that depend on the phenolic nature of the drug were studied. The methods described are suitable for the determination of oxyphenbutazone in control laboratories.

Experimental

Spectrophotometer

A Unicam SP 600 spectrophotometer was used.

Reagents

Hydrochloric acid, 6 N.
Sodium nitrite solution, 10% *m/V*.
Sodium hydroxide solution, 15% *m/V*.
Sodium carbonate solution, 1% *m/V*.
4-Aminoantipyrine, 2% *m/V solution in ethanol*.
Potassium hexacyanoferrate(III) solution, 4% *m/V*.

Procedures

Nitrous acid method

Weigh accurately about 100 mg of oxyphenbutazone, or its equivalent as crushed tablets or melted suppositories, into a small beaker. Add 20 ml of 0.1 N sodium hydroxide solution, mix thoroughly and transfer into a 100-ml calibrated flask. Wash the beaker with three 20-ml volumes of water, transferring each washing into the calibrated flask, and make up to volume with water. Filter, if necessary, and transfer 10 ml of the clear solution into another 100-ml calibrated flask and make up to volume with water. Transfer a 5-ml aliquot into a 25-ml calibrated flask and add 1 ml of 6 N hydrochloric acid followed by 2.5 ml of sodium nitrite solution. Allow to stand in a boiling water-bath for about 35 min. Cool to room temperature and make up to volume with 15% *m/V* sodium hydroxide solution. Measure the absorbance of the resulting solution at 380 nm against water as a blank. Calculate the concentration of oxyphenbutazone by reference to a calibration graph obtained by assaying suitable standards by the method described.

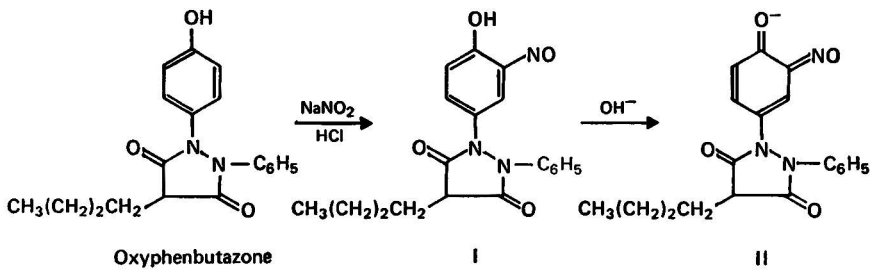

Fig. 1. Reaction of oxyphenbutazone with nitrous acid. I, *o*-nitroso derivative; and II, the quinoid form.

4-Aminoantipyrine method

The oxyphenbutazone sample and standard solutions are prepared and diluted in an identical way to that described in the nitrous acid method above. However, 10 ml of the diluted solution are transferred into the 25-ml calibrated flask. To this flask are then added,

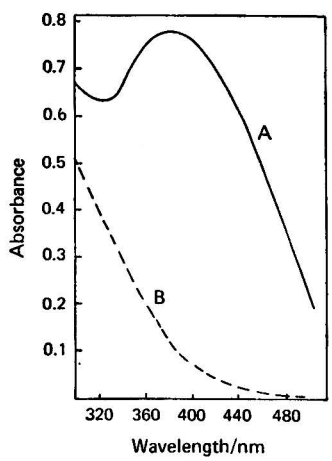

Fig. 2. Spectrum of oxyphenbutazone - nitrous acid reaction product in A, alkaline; and B, acidic media at a concentration of 0.025 mg ml^{-1}.

in sequence, 1.5 ml of sodium carbonate solution, 0.6 ml of 4-aminoantipyrine solution and 1 ml of potassium hexacyanoferrate(III) solution. Make up to volume with water and measure the absorbance at 500 nm against a reagent blank. Calculate the concentration of oxyphenbutazone by reference to a calibration graph obtained by assaying suitable standards by the method described.

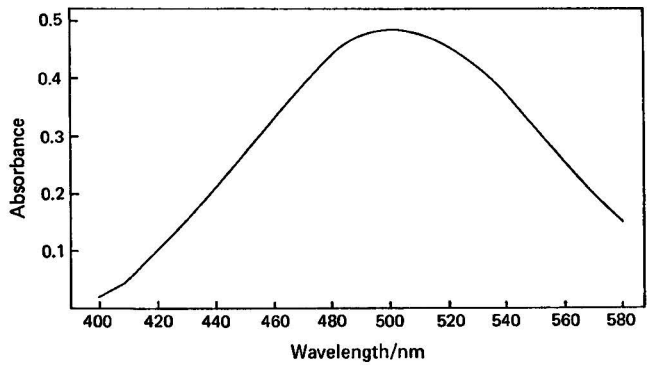

Fig. 3. Spectrum of oxyphenbutazone - 4-aminoantipyrine reaction product at a concentration of 0.0311 mg ml^{-1}.

Results and Discussion

Oxyphenbutazone is a phenol with a vacant *ortho*-position and can therefore react with nitrous acid to give the *o*-nitroso derivative,[5] as shown in Fig. 1.

Feigl[6] reported that the *o*-nitroso derivative (see Fig. 1), as the tautomeric oxime form, can yield metal chelates; this fact can be used to confirm experimentally that the above reaction pathway is correct. We succeeded in extracting the cobalt chelate of the *o*-nitroso derivative (**I**) using isobutyl methyl ketone. The reaction is affected by heating and it was found that the optimum temperature range is 70–100 °C; however, a boiling water-bath is recommended for simplicity. The colour reaches its maximum intensity after heating for 25 min and remains stable for up to 1 h. If the reaction medium (Fig. 2) is made alkaline, a bathochromic shift accompanied by a hyperchromic effect occurs, owing to rearrangement to the quinonoid form (**II**). Under these conditions Beer's law is obeyed over the range 4–24 $\mu g\ ml^{-1}$.

The second method proposed depends on the formation of antipyrine dyes as a result of the condensation of 4-aminoantipyrine with phenols in the presence of an alkaline oxidising agent.[7] Maximum absorption occurs at 500 nm (Fig. 3). The effects of different reagents on the reaction were studied. The optimum concentrations for sodium carbonate, 4-aminoantipyrine and potassium hexacyanoferrate(III) reagents were determined and incorporated

TABLE I

RECOVERY OF OXYPHENBUTAZONE FROM STANDARDS USING THE NITROUS ACID METHOD

Mass of oxyphenbutazone taken/mg	Recovery, %*
0.10	100.8
0.20	102.8
0.25	101.6
0.30	100.8
0.35	101.4
0.40	96.3
0.45	97.7
0.55	101.2
Mean ($P = 0.05$)	100.4 ± 1.8

* Results are the averages of two determinations.

into the method. The results obey Beer's law for oxyphenbutazone at concentrations of 8–48 μg ml^{-1} when the procedure described is used.

The mean value of $A^{1\%}_{1cm}$ obtained when the nitrous acid reaction is used is 310 with a coefficient of variation of 1.79%. The corresponding value for the 4-aminoantipyrine colour reaction is 154 with a coefficient of variation of 1.4%. The former method is therefore about twice as sensitive as the latter. The recoveries from spiked oxyphenbutazone samples using both methods are given in Tables I and II.

TABLE II

RECOVERY OF OXYPHENBUTAZONE USING THE 4-AMINOANTIPYRINE METHOD

Mass of oxyphenbutazone taken/mg	Recovery, %*
0.20	97.4
0.35	99.7
0.40	97.4
0.50	99.0
0.55	101.8
0.60	99.4
0.70	98.5
0.75	99.6
0.80	99.4
0.90	97.6
1.00	101.0
1.20	100.4
Mean ($P = 0.05$)	99.3 $\pm$ 0.9

* Results are the averages of two determinations.

Both methods were applied to the analysis of commercial preparations and the results are given in Table III. As the exact concentration of oxyphenbutazone in these preparations is unknown, the results in Table III were subjected to a paired comparison test.[8] The calculated values of Student's t for the results obtained using the BP method compared with results from the nitrous acid and the 4-aminoantipyrine methods were found to be 0.595 and 0.116, respectively; the corresponding tabulated value is 2.571. In this respect, there is no significant difference between the results of the two spectrophotometric methods and the titrimetric BP method as applied to commercial preparations. However, the proposed methods have the advantage over the titrimetric BP method that they can be used to determine microgram amounts; they also have the advantage over the spectrophotometric method of Sanghair *et al.*[2] that no preliminary hydrolysis is required. Phenylbutazone does not interfere in either of the proposed methods, permitting their application to the determination of oxyphenbutazone in biological fluids (this application is beyond the scope of the present paper); this is a further advantage over other methods.

TABLE III

APPLCATION OF THE PROPOSED METHODS TO THE DETERMINATION OF OXYPHENBUTAZONE IN PHARMACEUTICAL PREPARATIONS

Preparation and manufacturer	Stated oxyphenbutazone content, mg per tablet or suppository	Results, mg per tablet or suppository		
		NAM*	BP*	4AP*
Tandril tablet (Ciba)	100	100.38	97.32	98.60
	100	97.69	95.96	95.96
Oxyzone tablet (Nile Co.)	100	98.14	97.97	98.78
	100	96.15	97.69	96.77
Rhumaxin tablet (Alexandria Co.) ..	100	96.88	97.30	98.50
Rhumaxin suppository (Alexandria Co.)	250	240.75	243.25	241.25

* NAM = Nitrous acid method; BP = British Pharmacopoeia method; 4AP = 4-aminoantipyrine method.

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Received *June 1st*, 1979
Accepted *August 24th*, 1979

Method for the Determination of Phosphorus in Lipids and Lipid-containing Materials

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Keywords: *Phosphorus determination*; *phospholipids*; *alkali digestion*; *PTFE crucible*; *spectrophotometry*

The determination of phosphorus in lipids is usually preceded by the destruction of the organic matter by digestion with acids, such as sulphuric, nitric and perchloric, or by dry ashing. Both procedures are time consuming and digestion with perchloric acid, when applied to fatty acid esters, is a dangerous practice, as has rightly been pointed out by Wharton.[1] It was a violent explosion in the authors' laboratory, which occurred when digesting a soya bean oil sample with perchloric acid, that prompted the development of the procedure reported in this paper.

Experimental

Saponification of Phospholipids

The difficulty of saponifying phospholipids is well known. Heating lecithin with aqueous or ethanolic alkalis removes choline and fatty acids but the resulting glycerophosphoric acid resists the action of strong bases and is only slowly hydrolysed by strong acids. Even after 100 h of refluxing with 6 N hydrochloric acid the hydrolysis of glycerophosphoric acid is not complete.[2] However, it was found during this work that heating with an excess of sodium hydroxide at 180 °C results in a complete saponification of phospholipids in about 30 min and this discovery formed the basis of the method of phosphorus determination to be described. There remained the problem of selecting a suitable container for the proposed alkali treatment, as glass, porcelain and even stainless-steel crucibles were found to be unsatisfactory. Teflon was finally chosen as a suitable material for a crucible because it contains no phosphorus and resists the action of alkalis up to 250 °C.

Materials

Samples of crude soya bean oil were obtained from the manufacturers. They were degummed in the laboratory with water, neutralised with sodium hydroxide solution and washed. The phosphorus contents of the original and treated oils were determined, as were those of a commercial soya bean oil, lecithin, glycerophosphoric acid prepared as described by Power and Tutin[3] and blood plasma from Wistar rats.

Alkali Digestion Method

Reagents

All reagents should be free from phosphorus.
Sodium hydroxide solution, 50% m/m.
Ethanol, 96%.
Hydrochloric acid, concentrated.
Hexane.
Ammonium molybdate solution, 5% m/V.
Reducing agent. This consisted of 0.5 g of *p*-methylaminophenol sulphate (metol), 12.5 g of sodium hydrogen sulphite and 2.4 g of sodium sulphite dissolved in 100 ml of water and kept in an amber-glass bottle at 2 °C.

Apparatus

Crucible. This was of about 50-ml capacity, with 4.5 cm external and 3.5 cm internal diameters, prepared from a PTFE rod.
Stirring rod. This was made of PTFE, 1 cm diameter and 10 cm long.
Spectrophotometer.
Analytical balance.
Electrically heated oven.
Hot-plate.

Procedure

The size of the sample depends on its phosphorus content, which should be known approximately. If this content amounts to 0.01% or less, 2.5 g should be taken; with higher phosphorus contents the amount of sample should be correspondingly reduced or the final dilution increased. The sample is weighed in a PTFE crucible, 2 ml of 50% sodium hydroxide solution and 5 ml of 96% ethanol are added and the mixture is heated on a hot-plate, on which it is stirred occasionally with a PTFE rod until almost dry. The crucible is then placed in an oven and kept at about 180 °C for 30 min. After cooling, the contents are transferred into a 200-ml beaker and the crucible is washed with 15 ml of distilled water. The washings are added to the beaker, followed by 5 ml of concentrated hydrochloric acid, and the mixture is boiled for a few minutes in order to liberate the fatty acids and to convert pyrophosphate into orthophosphate. After cooling, 10 ml of hexane are added, the mixture is transferred into a separating funnel and the aqueous phase collected in a calibrated flask of 25-ml capacity, being diluted to volume with the water washings of the hexane layer. The solution is next filtered through filter-paper and 10 ml of the filtrate are pipetted into another 25-ml calibrated flask.

The molybdenum blue colour is developed by adding to the flask 0.5 ml of concentrated hydrochloric acid, 1 ml of the molybdate solution and 2 ml of the reducing agent, in that order, and making up to volume with distilled water. A blank is prepared in the same way, but without the addition of the molybdate solution. After 30 min the absorbance is read at 690 nm.

In order to obtain a standard graph, measured amounts of potassium dihydrogen phosphate containing, for example, 0.01, 0.05, 0.1, 0.15 and 0.2 mg of phosphorus are placed in 25-ml calibrated flasks and 10 ml of 10% sodium chloride solution are added; thereafter, reagents are added and a blank is prepared as described above. The addition of sodium chloride solution is intended to establish conditions similar to those obtaining during the actual determination. The amounts of phosphorus, measured in milligrams, are plotted against the absorbances. The graph obeys Beer's law.

Ashing method

For comparison, samples of crude soya bean oil, commercial lecithin and glycerophosphoric acid were ashed in the presence of magnesium oxide in a Vycor crucible.[4] The percentage of phosphorus was determined by using the procedure described in the preceding section.

Results and Discussion

The essential part of the proposed method is the alkali-digestion step, by means of which phosphorus is converted into water-soluble phosphate without the time-consuming destruction of the organic matter. The subsequent steps are carried out as described under *Procedure*; alternatively, any other recognised spectrophotometric method of phosphorus determination can be applied.

TABLE I

ABSORBANCES OBTAINED WHEN CONDUCTING ALKALI DIGESTION IN VARIOUS CONTAINERS WITHOUT PHOSPHORUS ADDITION

	Absorbance at 690 nm	
Type of container	Experiment 1	Experiment 2
Glass beaker	0.630	0.407
Stainless-steel crucible ..	0.190	0.210
Teflon crucible	0.000	0.000

Various reagents recommended for the development of the molybdenum blue colour have been tested during this work, *e.g.*, amidol (2,4-diaminophenol hydrochloride), sodium 1-naphthol-2-amino-4-sulphonate, hydrazine sulphate and metol (*p*-methylaminophenol sulphate). All of these reagents have been found to be satisfactory, metol being selected because of its low price, availability and good performance.

TABLE II

COMPARISON BETWEEN ASHING AND ALKALI-DIGESTION METHODS

	Phosphorus (average of 5 determinations), %	
Sample	Ashing	Alkali digestion
Crude soya bean oil A	0.07712	0.07705
Commercial lecithin	2.631	2.629
Glycerophosphoric acid	17.708	17.716

The heating of the samples with an excess of sodium hydroxide at 180 °C led to the use of a PTFE crucible, which can easily be turned on a lathe in any workshop. The use of other materials resulted in a notable contamination with phosphorus, as can be seen in Table I. The use of a silica or platinum crucible was obviously out of the question.

Results obtained when comparing the alkali digestion with the ashing procedure appear in Table II. For crude soya bean oil the results yielded by the two methods differed by 0.0007%, the difference being similar to the standard deviations for single determinations reported by Hvolby[5] when employing the ashing method already referred to.[4] This close agreement seems to indicate that alkaline digestion of phospholipids under the conditions specified in the present work is as effective a method as the ashing procedure.

The proposed method applied to crude, water de-gummed and neutralised soya bean oils gave the results shown in Table III.

TABLE III

PHOSPHORUS CONTENTS OF CRUDE AND TREATED SOYA BEAN OILS

Method: alkali digestion. Average of 5 determinations. Standard deviation: 0.68 p.p.m. of phosphorus.

	Phosphorus content in soya bean oils, p.p.m.		
Sample	Crude	Water de-gummed	Neutralised and washed
A	770.5	84.3	3.3
B	557.4	59.4	2.3
C	608.3	60.8	2.4
D	491.7	58.7	2.9

Although the method has been developed primarily for the determination of phosphorus in lipids, it can be applied to other organic materials if the colour of the resulting aqueous solution does not affect the absorbance readings. The procedure was employed, for instance, in the determination of the phosphorus in the blood plasma of experimental rats and gave values ranging from 14.9 to 20.3 mg per 100 ml of plasma, in good agreement with standard methods.

The main advantage of the proposed alkaline digestion is in the saving of time and no health or safety hazards are involved, as in some acidic digestion methods. An additional feature is the use of a PTFE crucible, which is easily manufactured, practically unbreakable and, according to the experience gained during the present work, does not show any signs of corrosion or deterioration after prolonged use.

The successful saponification of glycerophosphoric acid, which is reputedly highly resistant to the action of alkalis, may be tentatively explained as follows. In the usual saponification procedure, conducted in aqueous or alcoholic media, the two negative charges of the glycerophosphate anion exert a repelling effect on the negatively charged hydroxyl groups of the alkali. Under non-hydrous conditions this effect is suppressed and the elevated temperature of 180 °C facilitates the attack by the base on the ester linkage of the glycerophosphoric acid.

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Received *May 22nd*, 1979
Accepted *July 10th*, 1979

Gravimetric Determination of Zinc by Precipitation with Benzimidazole Directly and from Homogeneous Solution by Replacement of Zinc in a Complex with EDTA

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Keywords: *Zinc determination*; *gravimetry*; *precipitation with benzimidazole*; *zinc - EDTA complex*; *calcium replacement of zinc*

Benzimidazole has been described as a quantitative precipitant for the gravimetric determination of copper(II) and cobalt(II) in an earlier paper.[1] Zinc is another metal ion for which salt formation with benzimidazole has been reported,[2] but no attempt appears to have been made to apply this reagent to the determination of zinc. The present work was, therefore, undertaken with a view to determining zinc with benzimidazole by direct precipitation, and by precipitation from homogeneous solution. The latter technique was attempted mainly in order to avoid interferences.

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Experimental

All of the chemicals used were of analytical-reagent grade. A stock solution (0.02 M) of the benzimidazole reagent was prepared from the purified sample as has been described previously.[1] The zinc and calcium solutions were made from their chemically pure salts in doubly distilled water and were standardised by the ammonium phosphate and oxalate methods, respectively, before use. Suitable salts of other metals were used to furnish their ions in solution.

Different sets of experiments were performed. Firstly, zinc was determined at varying pH values and the pH conditions for its quantitative precipitation with benzimidazole were investigated. Secondly, determinations in this pH range were carried out with different amounts of zinc by use of the direct precipitation method and of precipitation from homogeneous solution by the replacement of zinc in a complex with EDTA. Interferences by metal ions, Pb^{2+}, Bi^{3+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+} and Hg^{2+}, were examined in both of the methods. The pH measurements were made using a Metrohm Herisau pH meter, Model E520, and the associated electrodes.

Investigation of the pH Conditions for Quantitative Precipitation

Portions (10 ml) of zinc solution (1 mg of zinc in 1 ml) were transferred into separate 400-ml beakers and to each one 30 ml of 0.02 M benzimidazole reagent solution were added (about double the amount required for a 1:2 molar ratio of the metal to the reagent). The solution was diluted to about 150 ml and the pH adjusted by dropwise addition of ammonia solution with constant stirring. The mixture was heated at 50–60 °C on a water-bath for about 30 min, cooled to room temperature and filtered through a weighed sintered-glass crucible of porosity G4. The precipitate was washed with 0.1% (*m*/*V*) reagent solution, three times with distilled water and finally with 50% ethanol. It was dried at 110 °C for 2 h and weighed (Table I). [Conversion factor of zinc to $(C_7H_5N_2)_2Zn$ = 0.2179.]

Procedure

Determination of zinc by direct precipitation with benzimidazole

To a solution containing 5–25 mg of zinc, add 15–75 ml of the reagent solution (nearly double the amount theoretically required), dilute to about 150 ml and slowly raise the pH, by dropwise addition of ammonia solution, almost to 8. Heat the solution, cool and filter it, and wash, dry and weigh the precipitate as has been described earlier (see Table II). Alternatively, the precipitation can be effected by the dropwise addition of the reagent solution after the pH of the solution has been adjusted almost to 8 with ammonia solution.

The interferences by Pb^{2+}, Bi^{3+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+} and Hg^{2+} with this method were examined and it was found that except for Ni^{2+}, all other cations interfered seriously in the determination. Nickel, even up to an equimolar concentration, did not interfere.

Determination of zinc in a solution containing zinc alone by precipitation from homogeneous solution

Place a 5–25-ml aliquot of zinc solution (5–25 mg of zinc) in a 400-ml beaker, add EDTA (disodium salt) solution in excess of the amount required to combine with all of the expected amount of zinc (30 mg of zinc requiring about 100 mg of EDTA), add 15–75 ml of the benzimidazole reagent solution (0.02 M), adjust the volume to nearly 150 ml, raise the pH to about 8 and heat the solution to 50–60 °C. Add 0.02 M calcium solution slowly with constant stirring until an amount rather greater than that which is equivalent to the excess of EDTA and the expected amount of zinc (or to the total EDTA) has been added. Following the addition of calcium solution allow the mixture to stand at room temperature with intermittent stirring for 2–3 h, and then filter, wash, dry and weigh as described earlier (Table III).

The amount of EDTA to be added is decided by the expected amount of zinc and not the exact amount of zinc. As EDTA should always be in excess, there is no harm if its amount exceeds the amount required by 30–40%.

On addition of the first drop of calcium solution the precipitation does not occur until about 30 s have elapsed. For the complete replacement of zinc by calcium a time of 2–3 h

is required. The calcium solution was prepared from an acetate salt but it was found, on experimentation, that solutions of calcium prepared from other salts were equally effective.

Determination of zinc in the presence of other metal ions by precipitation from homogeneous solution

The procedure is similar to that followed for zinc alone (above). A solution of zinc containing another metal ion (or ions) is treated with an excess of the EDTA and the reagent solution added in excess of the amount theoretically required for the metals anticipated. After adjusting the pH and heating the solution, the calcium solution is added in an amount rather more than that equivalent to the total EDTA (or the excess of EDTA and total metals expected) and the mixture is allowed to stand for 2–3 h, other conditions remaining the same. The analytical errors occurring in the presence of other metal ions (Pb^{2+}, Bi^{3+}, Cu^{2+}, Co^{2+}, Cd^{2+} and Hg^{2+}) did not exceed those occurring in their absence.

TABLE I

DETERMINATION OF ZINC BY DIRECT PRECIPITATION WITH BENZIMIDAZOLE AT VARYING pH VALUES

pH*	Mass of zinc taken/mg	Mass of precipitate obtained/mg	Calculated mass of $(C_7H_5H_2)_2Zn$/mg	Difference/mg	Error, %
6.5	10.0	33.9	45.8	−11.9	−25.980
7.0	10.0	45.6	45.8	− 0.2	− 0.438
7.5	10.0	46.0	45.8	+ 0.2	+ 0.438
8.0	10.0	45.9	45.8	+ 0.1	+ 0.219
8.5	10.0	45.7	45.8	− 0.1	− 0.219
9.0	10.0	46.0	45.8	+ 0.2	+ 0.438
9.5	10.0	41.5	45.8	− 4.3	− 9.390

* Average of three determinations each.

Results and Discussion

Results of the investigation of pH conditions (Table I) clearly indicate that the quantitative precipitation of zinc with benzimidazole can be effected in the pH range 7–9 and that determinations of zinc in solutions containing different amounts of zinc can be carried out satisfactorily in this pH range (Table II). The compound obtained was a white crystalline solid and had good filtration properties. It showed no loss of mass up to a temperature of 250 °C. However, the method has the disadvantage that a number of metal ions, *e.g.*, Pb^{2+}, Bi^{3+}, Cu^{2+}, Co^{2+}, Cd^{2+} and Hg^{2+}, which either form insoluble precipitates with benzimidazole or insoluble hydroxides in this pH range, interfere seriously.

TABLE II

DETERMINATION OF ZINC BY DIRECT PRECIPITATION WITH BENZIMIDAZOLE AT pH 7–9

Number*	Mass of zinc taken/mg	Mass of precipitate obtained/mg	Calculated mass of $(C_7H_5N_2)_2Zn$/mg	Difference/mg	Error, %
1	5.0	22.8	22.90	−0.10	0.438
2	12.0	54.8	54.96	−0.16	0.273
3	18.0	82.7	82.44	+0.26	0.315
4	23.0	105.0	105.34	−0.34	0.323
5	25.0	114.8	104.50	+0.30	0.262

* Average of three determinations each.

The results of determinations (Table III) carried out in the presence of EDTA by calcium replacement are encouraging. The compound precipitated in this way had a much larger particle size than that in the direct method. In addition, by using this method it was possible to determine zinc in the presence of those metal ions, which interfere in the direct precipitation method, *viz.*, Pb^{2+}, Bi^{3+}, Cu^{2+}, Co^{2+}, Cd^{2+} and Hg^{2+}. As the precipitation occurs only after about 30 s have elapsed following the first addition of calcium solution, it can be regarded as a case of precipitation from homogeneous solution.

TABLE III

DETERMINATION OF ZINC BY PRECIPITATION WITH BENZIMIDAZOLE FROM HOMOGENEOUS SOLUTION BY REPLACEMENT OF ZINC FROM AN EDTA COMPLEX (pH 7–9)

Number*	Mass of zinc taken/mg	Mass of precipitate obtained/mg	Calculated mass of $(C_7H_6N_2)_2Zn$/mg	Difference/mg	Error, %
1	5.0	23.0	22.9	+0.1	+0.438
2	10.0	45.6	45.8	−0.2	−0.438
3	15.0	68.9	68.7	+0.2	+0.292
4	20.0	92.0	91.6	+0.4	+0.438
5	25.0	114.1	114.5	−0.4	−0.349

* All the results are the average of five determinations.

There are two interesting aspects of the method. Firstly, calcium with a lower formation constant (log K = 10.7) is able to replace zinc from its EDTA complex, which has a higher formation constant (log K = 16.2) and secondly, only zinc is replaced and not the other metal ions. The replacement of zinc by calcium can be attributed to the strong tendency of the former to react with benzimidazole to give an insoluble product, the latter having no such tendency. This reaction can well be compared with the replacement of zinc by calcium in an EDTA complex during the precipitation of zinc sulphide in the presence of cobalt and nickel.[3] The replacement of zinc only and not the other metal ions by calcium can be explained partly on the basis of greater insolubility of the zinc complex and partly on the relatively weak nature of the zinc - EDTA complex in this pH range.

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Received *January 15th*, 1979
Accepted *July 26th*, 1979

Separation and Identification of Mixtures of Urea and Hydroxymethyl-substituted Ureas by Paper Chromatography

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Keywords: *Urea and hydroxymethylurea separation; paper chromatography*

The manufacture of urea - formaldehyde (UF) resins from aqueous solutions of urea and formaldehyde is a well established industrial process going back over 70 years. Although the broad outline of the chemistry of the process is well known, including the characterisation of many of the intermediate products, in general the reported work has not dealt with the quantitative aspects of the various intermediates produced during resin production.

The production of UF resins in commercial plants usually takes place in two stages: an initial reaction between urea and formaldehyde in aqueous media under alkaline conditions, followed by acidification of the reaction mixture. A series of condensation and addition reactions occur, resulting in the production of the UF resin. During the acidic stage the primary reactions are of the condensation type, producing methylene-linked species. The first condensation product was isolated in 1884[1] and later studies have provided data on additional reaction products[2,3] and also on the general reaction kinetics.[4,5]

This paper, however, is concerned with the separation and identification of the more important species present in the first stage of the process, *i.e.*, under alkaline conditions. At pH values between 7 and 11 the dominant reactions are additive and involve the production of hydroxymethyl derivatives of urea.

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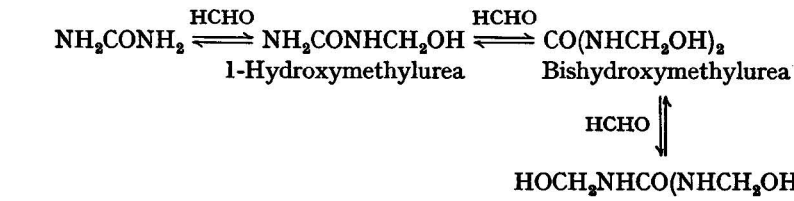

The predominant products are 1-hydroxymethylurea and bishydroxymethylurea, which were first isolated by Einhorn and Hamburger in 1908.[6]

Other methods, based on gas - liquid chromatography and ^{13}C nuclear-magnetic resonance spectrometry, for separating and identifying these species have been reported.[7,8] However, the technique described here is simple, requiring unsophisticated, inexpensive equipment.

Experimental

Reagents

AnalaR-grade reagents were used unless specified otherwise.

Urea.

Formalin.

Pyridine.

Chloroform.

Hydrochloric acid, concentrated.

4-Dimethylaminobenzaldehyde solution. Dissolve 1 g of 4-dimethylaminobenzaldehyde in acetone (100 cm^3).

1-Hydroxymethylurea. Prepared in the laboratory.

Bishydroxymethylurea. Prepared in the laboratory.

Procedure

A 10-μl volume of the test solution is applied as a 7 mm diameter spot on to a sheet of Whatman No. 1 chromatography paper. The small spot size is achieved by successive applications of sample followed by drying in a current of cool air. The spots are situated at least 110 mm from one end of the paper (this ensures that the mobile phase has achieved an even flow-rate before the samples are reached) and 40 mm apart (thus overcoming any lateral diffusion effects).

After the spots have been finally dried, the papers are developed by the normal descending technique in a sealed glass tank, held at constant temperature. Before use, a small amount of the developing solvent (pyridine - chloroform - water, 17 + 8 + 3) should be placed in the tank and the atmosphere allowed to equilibrate. The paper remains in the tank, until the solvent front has travelled a distance of 300 mm from the point of sample application (this takes about 4 h at 22 °C), then it is removed and rapidly dried in a current of cold air.

When the paper is thoroughly dry it is sprayed with 4-dimethylaminobenzaldehyde solution and then placed in an atmosphere saturated with hydrochloric acid vapour. After exposure for a few minutes the paper can be removed and any urea or urea derivative will be visible as a yellow spot.

The R_F values of urea, 1-hydroxymethylurea and bishydroxymethylurea are determined by applying the above procedure to pure solutions of the three species. Confirmation that the values are unaltered in the mixtures of the three species, plus formaldehyde, is obtained by eluting such mixtures by the given procedure.

Analysis of the reaction products of urea and formaldehyde at any given stage of the addition process is carried out quickly (*i.e.*, rapid removal of sample followed by immediate spotting and elution) to minimise disturbance of the species equilibria.

Results and Discussion

The R_F values of the three species studied increase with the increasing number of substituted hydroxymethyl groups. The R_F values were found to be 0.28 for urea, 0.39 for 1-hydroxymethylurea and 0.49 for bishydroxymethylurea. These values differ sufficiently to produce satisfactory separations in the 300 mm of solvent travel.

The principle by which the various species are separated is based on the number of hydroxymethyl groups present in the structure.[9] It is possible, under certain conditions of pH and temperature, to produce condensation products containing methylene-linked and methylene ether-linked species in addition to urea and the substituted ureas. These linked species can also contain hydroxymethyl groups and will not necessarily separate, therefore, from the urea and the hydroxymethyl-substituted ureas during the elution process. Fortunately, however, it is not difficult to determine when these condensation products have been produced because they are relatively insoluble in water and precipitate; the formation of precipitates, therefore, is an immediate indicator that the solution is not suitable for analysis by the method described in this paper.

The method described will, therefore, qualitatively analyse aqueous mixtures of urea and 1- and bishydroxymethyl-substituted ureas in the presence of formalin. However, more highly substituted ureas are also known to exist; the solvent system employed will separate these species, but they cannot be identified because the pure compounds have not, as yet, been isolated. In any event, under normal conditions trihydroxymethylurea and its higher analogues are rarely formed in more than trace amounts.

We have found the proposed procedure to be suitable, in most instances, for the analysis of mixtures of urea and formalin. The presence of methylene- or methylene ether-linked materials, which have been found in a small number of the mixtures, interferes with the analysis and thereby imposes certain limitations on the use of the method in the general urea - formaldehyde resin field.

Provided that the procedure given is rigidly adhered to, it has been found that this technique can also be used for quantitative determinations of urea, 1-hydroxymethylurea and bishydroxymethylurea in the presence of formalin. This can be achieved by accurate measurements of the final spot area, which are then related to previously prepared calibration graphs. We are at present developing such a method.

However, even as the method stands at present, it can still be used to give a visual indication of the relative proportions of each of these species in different urea - formaldehyde mixtures.

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Received *August 2nd*, 1979
Accepted *August 29th*, 1979

Improved Method for Detecting Microgram Amounts of Iron

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Keywords: *Iron test*; *iron catalysis*

Based on our work on the iron(III)-catalysed peracetic acid oxidation of phenol and catechol to *cis,cis*-muconic acid (MA),[1] we have devised a simple and sensitive new test for iron. The test described in this paper, which we refer to as the "MA test for iron," has the advantage over previously reported tests of being essentially interference free whereas other methods are sensitive to several interfering ions. In addition, amounts of iron down to

about 0.05 μg can be detected, making the method more sensitive than most previously described tests.[2]

The MA test is based on the ability of iron to promote the formation of MA in the peracetic acid oxidation of phenol and/or catechol in acetic acid solvent. MA, a white solid, is insoluble in the medium and precipitates from solution as it is formed. No MA precipitates from solution in the absence of iron(III) or copper(II). Copper(II) is also capable of promoting the formation of MA but is much less efficient, requiring relatively large amounts of copper(II).[1] An MA precipitate is not formed in the presence of the following metal species: aluminium(III), zinc(II), nickel(II), cobalt(II), manganese(II), vanadium(0), silver(I), sodium(I), mercury(II), iron(II), chromium(III), tungsten(VI) and titanium(IV). The ability of a metal to promote the formation of MA is related to how strongly it binds catechol and to its reduction potential. Only metals that form exceptionally stable complexes with catechol and are easily reduced promote MA formation.[1,3] The only metals known to fulfil these requirements are iron(III) and copper(II), with the former meeting the criteria better than the latter.

The MA test can be carried out using phenol or catechol as the testing reagent. The test employing catechol has the advantage of being faster whereas the test with phenol is more sensitive.

Experimental

Materials

It is important that only analytical-reagent grade materials low in iron be used as others may contain sufficient iron impurities to give a positive test. The acetic acid was Matheson Scientific ACS reagent. The peracetic acid was obtained from FMC Corp. as a 40% *m/m* solution. Catechol was supplied by Fisher (certified). The phenol used was from Mallinckrodt, analytical-reagent grade, or MCB, reagent grade. Sources of iron included the following: $Fe(OAc)_2OH$ (City Chemical Corp., purified); iron filings (MCB, degreased); $FeCl_3.6H_2O$ (MCB, ACS reagent); and $Fe(OAc)_2$ (Ventron).

MA Test Using Phenol

Mix 0.5 g of phenol, 2.0 g of acetic acid and 0.1–0.5 ml of the aqueous solution to be tested in a small test-tube (a solid can be used directly). Add a solution consisting of 1.0 g of acetic acid and 3.0 g of 40% peracetic acid. Mix well and allow to stand at room temperature. The formation of a white precipitate of MA (melting-point 180 °C with decomposition)[1] within 24 h indicates a positive test. The limit of detection is 0.05 μg of iron. Copper(II) interferes. At least 115 μg of copper(II) must be present in 0.1 ml of test solution in order to obtain a positive result. This amount of copper(II) can easily be detected by using concentrated aqueous ammonia or other simple tests.[2]

MA Test Using Catechol

To a magnetically stirred solution of 5 ml of an aqueous solution containing iron(III) and 15 ml of cold (5 °C) 40% peracetic acid in a 50-ml Erlenmeyer flask add, at a rate of approximately 1 drop per minute, 10 ml of an acetic acid solution containing 3 g of catechol. A white precipitate of MA will appear in 1–4 h depending on the amount of iron present. For example, with 850 μg of iron(III) the precipitate appears in less than 1 h whereas with 2 μg the precipitate appears in about 4 h. Solids can be used directly as in the test with phenol. The limit of detection is 0.5 μg. Copper(II) interferes.

Results and Discussion

The MA test is extremely sensitive. The catechol testing procedure can detect amounts of iron as low as 0.5 μg whereas the phenol method is sensitive to 0.05 μg. A comparison of the MA test with other common tests for iron is presented in Table I. It is clear that the MA test is superior to all other tests for iron. The source of iron(III) is not important. Thus, acetate, chloride, sulphate and nitrate salts of iron(III) give positive tests, as does iron metal, which is presumably oxidised to iron(III) by peracetic acid before MA formation. Iron(III) can be detected in the presence of any of the previously listed metals, demon-

strating that they do not interfere with the catalytic effect of iron in this reaction. Solvents are not necessary. For example, iron filings can be added directly to the test solution. Non-aqueous solutions can be used, but one must keep in mind that some organic solvents are susceptible to oxidation by peracetic acid, and thus may divert it from the oxidation of phenol or catechol giving rise to a false negative result.

TABLE I

COMPARISON OF MA TEST WITH OTHER COMMON TESTS FOR IRON(III)[2]

Test	Limit of identification/ μg	Interfering ions and/or conditions
MA	0.05	Cu(II)
Hexacyanoferrate(II) ..	0.1	Metal salts that give coloured precipitates with $K_4Fe(CN)_6$. Anions that form complexes with Fe(III) (F^-, oxalate, etc.)
Ferron	0.5	Coloured ions or strong oxidising agents; pH must be maintained at 2–3.5; Cu(II), Co(II), chromates and vanadates interfere with modified method using ion-exchange resin
Thiocyanate	0.25	Co, Ni, Cr and Cu salts; F^-
Tiron	0.05	Ti, Cu and Mo salts

Iron in the 0, +2 and +3 oxidation states can be detected. It is not possible to differentiate iron(0) from iron(III), but it is possible to differentiate iron(II) from iron(III) under conditions where only one is present. As iron(II) is known to decompose peracetic acid solutions rapidly,[1] it will not promote the formation of MA under the testing conditions described above. If rapid and noticeable decomposition of the peracetic acid testing solution is observed with little or no oxidation of phenol, then the presence of iron(II) may be indicated. To confirm, treat a small portion of the test solution with an appropriate oxidising agent such as hydrogen peroxide and then perform the MA test again. A negative initial test followed by a positive test after oxidation leads to the conclusion that iron(II) was initially present.

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Received *July 4th*, 1979
Accepted *August 8th*, 1979

Communication

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, 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.

Application of Transition-metal Salts as Ion-pair Reagents in the Liquid Chromatography of Dithiocarbamates

Keywords: *Dithiocarbamate analysis*; *liquid chromatography*; *ion-pair reagents*; *transition-metal salts*

N-Alkyl- and *NN*-dialkyldithiocarbamates and their salts and disulphides are widely used as fungicides, rubber vulcanisers and pharmaceuticals. As part of a study of the degradation of the cyclic fungicide dazomet (3,5-dimethyltetrahydro-1,3,5-thiadiazine-2-thione), we wished to analyse *N*-methyldithiocarbamate (MDTC), one of the primary decomposition products.[1,2] Existing techniques based on ultraviolet spectroscopy, total degradation to carbon disulphide and methylamine and thin-layer, paper or gas - liquid chromatography[3] either could not differentiate MDTC from other components of the reaction or could not be used to monitor the other components in the same analysis.

Although reversed-phase liquid chromatography could be used to analyse dazomet and neutral degradation products, MDTC was not retained even with aqueous phosphate buffer (pH 5) as solvent. Eluents with a lower pH could not be used to suppress ionisation as dithiocarbamates are very unstable at pH below 4.[4] The ion-pair reagents tetrabutylammonium hydroxide and cetrimide were tried, but appeared to result in the degradation of MDTC.

As the transition-metal salts of a number of dithiocarbamates have been successfully separated using liquid chromatography,[5] we investigated the use of transition-metal ions as ion-pair reagents. The results of our study using the direct injection of the sample into an eluent containing transition-metal ions suggest that this approach could be a versatile and powerful method for the analysis of dithiocarbamates.

Experimental and Results

Samples of 10 μl were separated on a 5-μm Hypersil-ODS column (10 cm $\times$ 5 mm i.d.) using a Waters Associates 6000 pump and an ALC 202 detector at 254 nm, with a solvent flow-rate of 1.5 ml min^{-1}. The samples of the sodium salts of MDTC and *N*-ethyldithiocarbamate (prepared according to Klopping and Van der Kerk[6]), *NN*-dimethyldithiocarbamate (Aldrich) and *NN*-diethyldithiocarbamate (Sigma) were prepared as solutions in methanol - water (7 + 3). Analytical-reagent grade nickel sulphate and cobalt nitrate were used as ion-pair reagents at a concentration of 0.1%

The capacity factors of the dithiocarbamates and some related thiuram disulphides with the different metal ions are given in Table I. The peaks were readily detected without background interference from the metal ions in the solvent. The efficiency of the column was unchanged in comparison with unpaired eluents, suggesting that complex formation was rapid.

As the dazomet degradation studies were carried out in phosphate buffer, it was necessary to use methanol - phosphate buffer (pH 5) (3 + 7) instead of water, to prevent precipitation of transition-metal phosphates on injection. Using these conditions with 0.1% cobalt(II), the peak-height response of MDTC was linear from 100 to 1000 μg ml^{-1}.

An attempt to analyse disodium ethylenebisdithiocarbamate was unsuccessful, probably because it forms polymeric metal salts.

TABLE I

CAPACITY FACTORS OF DITHIOCARBAMATES WITH TRANSITION-METAL ION-PAIR REAGENTS

	Solvent			
		Methanol - water (6 + 4)		
	Methanol - water (7 + 3), ion pair 0.1% Ni(II)	Ion pair 0.1% Ni(II)	Ion pair 0.1% Co(II)	Methanol - buffer* (3 + 7), ion pair 0.1% Co(II)
Dithiocarbamate (Na salt)—				
N-Methyl (MDTC)	0.57	1.25	0.71	11.8
N-Ethyl	1.28	3.37	2.71	
NN-Dimethyl	1.57	4.12	3.85	
NN-Diethyl	8.28	32.1	—†	
Thiuram disulphide—				
NN'-Tetramethyl (thiram)	0.85	1.25	1.57	
NN'-Tetraethyl (disulfiram)	3.71	11.8	12.7	

* 0.05 M phosphate buffer adjusted to pH 5.
† Peak not observed.

Discussion

Little previous use has been made of metal ions as reagents in liquid chromatography, with the exception of the interaction of silver(I) and olefinic bonds, to give a complex with increased polarity (*e.g.*, ref. 7). In this study, the transition-metal ions in the solvent form a neutral non-aqueous soluble ion-pair complex with the injected dithiocarbamates, enabling them to be resolved from the solvent front and to be eluted under similar conditions to the corresponding disulphides.

Although only nickel(II) and cobalt(II) metal ions have been studied in this way, the work by Schwedt[5] on the separation of pre-formed complexes of diethyldithiocarbamate suggests that lead(II), mercury(II), copper(II) (and possibly others in less polar solvents) should act in a similar way. As each of these complexes has a different capacity factor, it should be possible, by selection of the ion-pair reagent, to adjust the retention of the complex so that it can be resolved from other components of a mixture. The order of elution of the complexes of lead, nickel, cobalt, copper and mercury was the same for diethyldithiocarbamate and tetramethylenedithiocarbamate,[5] but does not agree with the present results in which cobalt(II) complexes are eluted before nickel(II) complexes. Other metal ion salts of diethyldithiocarbamate, such as iron(III), molybdenum(II), chromium(VI) and vanadium are reported[5] to give many peaks on direct injection and would probably be unsuitable for pairing.

Using cobalt(II) as the ion-pair reagent, this technique was applied to the study of dazomet and permitted the determination of dazomet and its degradation products, MDTC, methyl isothiocyanate and *NN*-dimethylthiuram disulphide, in the same run.[8]

Further studies into the scope and applications of this technique are in progress.

We thank the SRC for a Studentship to R. L. M.

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Received *December 5th*, 1979

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Book Reviews

QUALITATIVE ORGANIC ANALYSIS. Revised Edition. By WILLIAM KEMP. Pp. xvi + 183. London, New York, etc.: McGraw-Hill. 1979 (first published 1970). Price £4.85 (soft-back).

The great emphasis on molecular spectroscopic techniques in organic chemistry has in recent years drastically changed the methods taught for identifying organic compounds. In some instances these changes have been to the detriment of the chemical aspects. The author makes it clear that the chemical and spectroscopic techniques are not mutually exclusive and that a proper balance between the two approaches is important to laboratory teaching. The book aims to inculcate an iterative approach, as used by practising chemists, to give a sound grounding in qualitative organic analysis, none of which has to be "unlearnt" in later practice.

The approach used is as follows. General chemical tests are used to assign a compound to a class and the chemical and infrared evidence is used to deduce the probable functional group(s) in an unknown compound. The infrared spectrum is then examined carefully for the presence of functions that have few distinguishing chemical reactions. Methods are described for the preparation of derivatives and tables of physical properties for series of compounds are given. Students are encouraged to use reference books and spectral collections, together with any other spectroscopic data they can obtain. Guidance is given to the interpretation of NMR, electronic and mass spectra. The book pays attention to laboratory safety and concludes with a discussion of the chemistry of class reactions and the separation of mixtures.

This is an excellent teaching text, which well supports the view that qualitative organic analysis is a vehicle for learning about organic compounds, their chemistry and their spectroscopy.

D. THORBURN BURNS

PHYSICOCHEMICAL APPLICATIONS OF GAS CHROMATOGRAPHY. By RICHARD J. LAUB and ROBERT L. PECSOK. Pp. xxiv + 300. Chichester, New York, Brisbane and Toronto: John Wiley. 1978. Price £16.60; $33.

The role of gas chromatography as an analytical technique, by the very nature of the tremendous success it has enjoyed for over two and a half decades now, has overshadowed all other possibilities and opportunities for the technique. However, without being patronising there always has been a relatively small but dedicated band of physical chemists who have expounded, lectured upon and clearly demonstrated within the chemical literature these other capabilities, especially those concerned with the accumulation of thermodynamic data. The present book is an attempt to present the considerable advances that have been made in the last few years to a wider audience. However, it is more than that, as the book places the role of physico-chemical measurement via gas chromatography in an historical context, where the technique is also compared with more conventional methods of acquiring the same information. Of particular interest to the analytical scientist is the summary of retention parameters, how they are measured and a critical appraisal of commercial and "home-made" equipment for such work. Here we have the "dynamic" *versus* "static" arguments very well presented.

As to be expected, Part II of the book is concerned with the determination of virial coefficients, and the intriguing use of chemically active mobile phases and complexation and adsorption phenomena. Here the analyst should be able to find proposals and models for the observations often easily made but difficult to explain.

Parts III and IV become a little more esoteric in that there is presented a discussion of the possibilities of gas chromatography for studies of kinetics and for the determination of the physical properties of pure compounds (*e.g.*, vapour pressure and heats of vaporisation and solution). However, even here the role of the gas density balance (that paradox of holes drilled into a copper block, which are then almost all carefully re-sealed) is presented plus the appropriate theory; hardly advances, but it raises such nostalgic memories.

The book is not easy going—it is physical chemistry at its most intriguing but nonetheless very rigorous for that. I am sure a physical chemist may view the book in a different light and suggest

that this model or that theory has been missed or badly presented. To an analytical scientist it is a book that describes an area into which we rarely incur, often because of our incompetence or impatience to handle equations and understand the fundamental parameters being discussed. The loss is ours, but often we seem more concerned with recoveries, detection limits and the reproducibility of methods using gas chromatography, and the topics within the book should be of interest to all workers using a gas chromatograph who think beyond the injection port and the recorder chart.

The book is well produced, with clear, understandable tables and diagrams. The circle of readers will doubtless be small as the authors are most likely preaching to the converted; they deserve better than that for the effort they have made. G. NICKLESS

PRÄ-CHROMATOGRAPHISCHE MIKROMETHODEN. By WOLFGANG DÜNGES. *Chromatographische Methoden.* Pp. 312. Heidelberg, Basle and New York: Hüthig Verlagsauslieferung. 1979. Price DM36.80.

This is a very portable (approximately 21 × 15 cm) soft-backed gem of a book, packed with a wealth of facts and detail, which will be of use not only to the bilingual biomedical chromatographer but even to an insular English analyst.

It details pre-chromatography micro-scale methods in biomedical and related fields. Its five parts comprise a general introduction, fundamental details, biomedical analyses, particular chromatographic experiments, and an appendix indicating literature available, explanations of reactions given earlier in the book, and providing references and tables of data relating to specific chapters (there is also a useful index). These five parts are subdivided into 24 chapters, or sections, each with sub-sections.

Methods, together with descriptions of micro-apparatus used, are given for extractions, for reduction of sample volume, derivatisation and final determinations. The principal chromatographic techniques are covered, namely, gas - liquid, thin-layer and high-performance chromatography, and the various types of substrate or column are discussed. A number of experiments are detailed stepwise: commencing (for example) with a few microlitres of blood—the stages of extraction, drying, micro-refluxing and derivatisation, and finally completion with the appropriate technique.

Numerous figures are provided, showing actual chromatograms produced, structural formulae, standard graphs and drawings of the micro-apparatus required—for example, including items such as small condensers, micro-refluxing equipment and more specialised apparatus for concentration procedures.

Mass spectrometry and ultraviolet and infrared spectroscopy are also mentioned, where applicable. Examples of the materials referred to include barbiturates, pesticides, alkaloids, antihistamines, tranquillisers and antibiotics, all of which are present in trace amounts only, and figures are given for accuracy and precision in quantitative work.

The book represents good value for money and purchase is recommended. D. SIMPSON

STATISTICAL METHOD IN BIOLOGICAL ASSAY. Third Edition. By DAVID J. FINNEY. Pp. xii + 508. London and High Wycombe: Charles Griffin & Company Ltd. 1978. Price £19.

For most practising analysts the statistical evaluation of results of quantitative assays is confined to the evaluation of new methods, particularly when examined by collaborative assay. It is generally accepted that in chemical and physical assays experimental conditions can be so specified as to ensure that determinations can be carried out that are precise and accurate. Occasionally, when conditions cannot be defined adequately, a simultaneous comparison against a reference material is necessary, as is the case in current pharmacopoeial assays of penicillins. In routine practice, reliance is often put on a single determination. Many laboratories will perform assays in duplicate, but it is rarely, if ever, possible to evaluate the precision of the assay of a single sample by a statistical examination of the assay data.

Bioassayists, quantitating an active substance by measurement of the response it produces in a living organism, must use a comparative assay. Because of the inherent variability of living organisms, a sufficient number must be used to ensure that the effects of uncontrolled variables in organism - environment interactions are distributed in a random fashion to all dose levels of

the unknown and of the standard preparation. A single assay is usually based on a large number of observations and, in order to extract a quantitative conclusion, statistical evaluation of the data is necessary.

Both the British and European Pharmacopoeias prescribe methods of assay for antibiotics, hormones and immunological products that require such statistical analysis and many analytical chemists have been confronted with the need to evaluate biological assays without prior experience or training in biometrics or statistics. The European Pharmacopoeia contains an Annexe relating to the statistical analysis of bioassay, but it is of necessity, with limitations of space, superficial. The general principles involved are stated and illustrated by reference to examples of different pharmacopoeial assays. The structure or design of a specific bioassay is not, however, defined in detail and the bioanalyst is allowed to vary the number of replicates, number of doses, size of dose interval, etc. In many instances, therefore, the guidance given in the pharmacopoeia is not adequate. For many years those in difficulty have turned to "Finney" for assistance; his textbook has been the standard work in the field of bioassay for over 25 years and few bioassayists in this and other countries have not drawn benefit from this source: assistance in understanding the principles of the problems involved, as well as in facilitating calculation of the desired statistics. The Second Edition was largely a re-editing of the first, but this Third Edition has been re-written in particular to take account of the major advances that have occurred in the availability and use of computers. Even the smallest laboratory can now afford a computer that allows the programmed calculation of an analysis of variance of a parallel line assay and the calculation of its confidence limits.

Radioimmunoassays, whilst not bioassays, share many of their problems. They perhaps form a bridge between biological and chemical assay. The book now includes practical guidance on their statistical design and interpretation, which could be useful to those involved in more strictly chemical assays of a similar nature.

The publication of the Third Edition is most appropriate at this time when the Second Edition of the European Pharmacopoeia is to be published without a statistical annexe. Although it is the stated intention to correct this deficiency in the European Pharmacopoeia in a future revision, this is unlikely to be effected for several years. With the new edition of "Finney" available, this omission from the European Pharmacopoeia should create no problems for English-speaking analysts.

J. W. LIGHTBOWN

VOGEL'S TEXTBOOK OF QUANTITATIVE INORGANIC ANALYSIS INCLUDING ELEMENTARY INSTRUMENTAL ANALYSIS. Fourth Edition. Revised by J. BASSETT, R. C. DENNEY, G. H. JEFFERY and J. MENDHAM. Pp. xxxii + 925. London and New York: Longman. 1978. Price £14.

In the 40 years since the first edition of A. I. Vogel's "Quantitative Inorganic Analysis" appeared, many generations of students have become familiar with the characteristic format and contents of the text. It has provided them with a convenient and comprehensive (if not always critical) source of information, which they have carried with them into their subsequent chemical careers and into analytical laboratories throughout the UK and Commonwealth. In this respect, the late Dr. Vogel provided a valuable service to the chemical community. Of course, like all successful authors, he has had his critics, but few would deny his books their almost unassailable position as student textbooks.

This new (and first posthumous) edition has been revised by four of Vogel's former colleagues who have deliberately set out to maintain the traditional features of the earlier editions. Obviously, the many significant developments over recent years, particularly in the area of instrumental methods, have had to be incorporated into the present text without unduly enlarging the book as a whole. This has been achieved largely at the expense of the more traditional methods of gravimetry and titrimetry, which many believed occupied excessively large sections of the earlier editions. The text is now divided into seven major parts, comprising 23 chapters, and dealing in turn with Fundamental Principles, Apparatus and Techniques, Errors and Sampling, Separative Techniques, Titrimetry and Gravimetry, Electroanalytical Methods, Spectroanalytical Methods, and finally Thermal Methods. Several Appendices containing useful data and information are included at the end of the book.

By and large, the treatment accorded to all these major topics is good, and of sufficient depth to give the average reader a proper understanding and appreciation of the subject matter. The

text of the new material is strengthened considerably by the inclusion of references and appropriate bibliography. Nomenclature has been updated and although SI units have been introduced throughout the text, the authors have had the courage to retain the concept of "equivalence" and hence "normality" in the section dealing with titrimetry. This is such an integral part of titrimetric analysis that much of its value as an analytical method is lost if concentrations have always to be expressed in moles per cubic decimetre—a fact that the SI protagonists seem unable to appreciate.

There are many commendable features in this new edition that will help it to retain the enviable following that earlier editions of Vogel undoubtably had amongst college students and practising analysts. Not the least of these is that a more balanced and critical text is now presented.

W. I. STEPHEN

STABILITY CONSTANTS OF METAL-ION COMPLEXES. PART B. ORGANIC LIGANDS. Compiled by DOUGLAS D. PERRIN for the International Union of Pure and Applied Chemistry Analytical Chemistry Division Commission on Equilibrium Data. *IUPAC Chemical Data Series—No.* 22. Pp. viii + 1263. Oxford, New York, Toronto, Sydney, Paris and Frankfurt: Pergamon Press. 1979. Price $150.

Users of "Stability Constants of Metal-ion Complexes" (Chemical Society Special Publication No. 17) will welcome this latest supplement that now brings the literature coverage, for organic ligands only, up to 1973 (a separate supplement on inorganic ligands is still in preparation).

This formidable task undertaken by the Commission on Equilibrium Data, Analytical Chemistry Division of IUPAC is part of a continuing programme to up-date, by means of supplementary volumes, the First Edition of "Stability Constants," which appeared in 1964. This (second) supplement covers the period 1969–73, although some data from literature before 1969, omitted from the 1964 Tables and its first supplement (1971), are now included. The data are presented as in the earlier volumes and likewise no attempt has been made to provide critical assessments of the individual constants or to comment on the accuracy of the published results, although where authors have given some estimate of the limits of error in their data, these are quoted. Hence, the value of the tables lies not so much in the availability of stability-constant data, but in the provision of a convenient source of reference to published work on metal complexes.

Since the publication of the 1964 Tables, workers in many areas of chemical research have greatly valued these compilations of equilibrium data on metal-ion complexes. Dr. Perrin and his colleagues who have undertaken this work on behalf of IUPAC deserve the grateful thanks of the chemical community. Regrettably, each supplement is larger than its predecessor, and escalating costs of printing have made the price of this volume well beyond the reach of private individuals and even smaller libraries. Nevertheless, this is a volume that should be as readily available to researchers as the volumes it supplements.

W. I. STEPHEN

QUANTITATIVE MASS SPECTROMETRY IN LIFE SCIENCES II. PROCEEDINGS OF THE SECOND INTERNATIONAL SYMPOSIUM, HELD AT THE STATE UNIVERSITY OF GHENT, JUNE 13–16, 1978. Edited by A. P. DE LEENHEER, R. R. RONCUCCI and C. VAN PETEGHEM. Pp. x + 501. Amsterdam, Oxford and New York: Elsevier. 1978. Price $48.45; Dfl109.

Progress in the development and application of sophisticated methods of analysis has been greatly assisted by informal discussions at small international meetings between specialists from different scientific disciplines. Although the formal lectures at such meetings are occasionally ephemeral, the frank discussions engendered help to spread detailed information on new procedures and, by drawing attention to practical problems, they encourage the introduction of more reliable instrumentation. Quantitative mass spectrometry in the life sciences, which was the subject of a successful symposium held in Ghent, at which the 5 Plenary Lectures and 41 communications reproduced in this volume were presented, is an important analytical technique that is dependent on full co-operation between experts in mass spectrometry, chromatography and computer technology on the one hand and practising biochemists and clinicians on the other.

The Plenary Lectures were presented by internationally known mass spectroscopists and clinical chemists, and they contain much useful advice on new methods. L. Siekmann surveys isotope dilution - mass spectrometry of steroid hormones and discusses its value as a routine method in clinical chemistry, while S. P. Markey, A. J. Lewy and R. W. Colburn describe uses of positive and

negative ion chemical ionisation mass spectrometry in studies of biogenic amine metabolism. Applications of gas chromatography - chemical ionisation mass spectrometry in the quantitative analysis of commonly abused drugs in physiological fluids are discussed by R. L. Foltz. H.-R. Schulten and W. D. Lehmann review applications of field ionisation and field desorption methods in the determination of organic compounds and metal cations in biological samples; special attention is given to preliminary work-up procedures. Methods for reducing errors associated with sampling, extraction, derivatisation and adsorption on glassware and criteria for selecting good internal standards are covered by B. J. Millard. He makes the point that a simple homologue of the compound of interest usually forms an adequate internal standard and that it normally has an advantage over stable-isotope labelled analogues in being more readily available.

The communications, which describe recent applications of quantitative mass spectrometry in drug metabolism, clinical chemistry, biochemistry, toxicology and environmental hygiene, contain much previously unpublished information. They fall into four subject groups, namely, topics of general interest including instrumentation and factors that affect accuracy and precision, quantitation of endogenous (physiological) substances, quantitation of drugs and drug metabolites and quantitation of other exogenous substances in biological fluids and tissues. The experimental procedures described involve the use of supplementary techniques, such as off-line high-performance liquid chromatography, glass capillary gas chromatography and negative ion chemical ionisation.

The volume, which has been printed by photographic reproduction of typescript and does not contain a detailed subject index, provides an instructive, state-of-the-art survey of biochemical and clinical applications of quantitative mass spectrometry. It has much to interest both the tiro and the experienced worker.

J. E. PAGE

SUGAR ANALYSIS. OFFICIAL AND TENTATIVE METHODS RECOMMENDED BY THE INTERNATIONAL COMMISSION FOR UNIFORM METHODS OF SUGAR ANALYSIS (ICUMSA). Edited by FERDINAND SCHNEIDER. Pp. xiv + 265. Peterborough: ICUMSA. 1979. Price £12.

This book is the Second Edition of a collection of "official" and "tentative" methods of analysis recommended for use in the sugar industry by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA). Since the First Edition was published in 1964, five further sessions of ICUMSA have been held, and it is not surprising that many changes in analytical procedures have been recommended since then. For this reason, the collation of all these changes into one volume will be especially welcomed by all analysts concerned with the analysis of sugars.

In this edition, "official" and "tentative" methods, up to and including those adopted at the 16th Session held in 1974, are given as full method descriptions. In order to incorporate the very latest recommendations, the amendments proposed at the 17th Session (1978) are included also as postscripts at the end of the methods affected. The subject matter is substantially the same as in the previous edition, but it is pleasing that SI units have been adopted and the completely revised order of presentation is to be preferred, in my opinion.

The book is presented in three parts, dealing with general methods, special methods and tables. As would be expected, over three quarters of the book is devoted to general methods, covering such topics as sucrose and polarisation, reducing sugars and various other saccharides, inorganic and organic non-sugars, dry substance-water, physical characteristics and microbiological tests. Additionally, sections describing specifications and tolerances for reagents and laboratory glassware are included. Part II is concerned particularly with the examination and evaluation of white sugar, raw sugar, bone char and carbons. Part III contains tables relating refractive indices at 20 and 27 °C and density of pure sucrose solutions with concentration.

The detailed method descriptions are clearly presented in a standard format and, wherever possible, are accompanied by appropriate references which should be useful to readers wishing to gain background information. Unfortunately, no data concerning reproducibility or precision of the methods are given, but this information was omitted deliberately as reliable statistical information was generally unavailable.

The book is well produced; very few typographical errors were detected, but a few pages having a heavy print intensity in my copy were mildly irritating to read. By current standards, it is reasonably priced and should be regarded as an essential reference work to analysts engaged in this field.

A. D. INCE

DENSITOMETRY IN THIN LAYER CHROMATOGRAPHY. PRACTICE AND APPLICATIONS. Edited by JOSEPH C. TOUCHSTONE and JOSEPH SHERMA. Pp. xvi + 747. New York, Chichester, Brisbane and Toronto: John Wiley. 1979. Price £21; $39.55.

To most analytical chemists, particularly those concerned with organic materials, thin-layer chromatography (TLC) needs little introduction. Simplicity of operation, low cost of the basic equipment, wide applicability, facility for side-by-side comparison of samples and the ability to yield semi-quantitative results by simple visual inspection are features that have led to the adoption of TLC as an essential technique in many laboratories. The massive bibliography that TLC has acquired since it was introduced on a firm practical foundation 20 years ago is ample evidence of its continuing value.

The chromatographic resolution attainable on conventional or high-performance TLC plates is adequate for many purposes, but problems arise in raising the quantitative performance to a level commonly expected of sound, reliable analytical methods. Numerous approaches to providing a fully quantitative finish in TLC have been made, perhaps the most promising being *in situ* densitometry by means of optical transmittance, reflectance, fluorescence or fluorescence-quenching measurements on the prepared chromatogram.

This book, apparently the first to be devoted entirely to densitometry in TLC, is opportune and to be welcomed. After a brief historical introduction, an important chapter is devoted to theoretical aspects of optical measurements in the context of scattering media and to a discussion of the Kubelka-Munk theory. This chapter is not the easiest to assimilate, but could be regarded as essential reading for those seriously committed to densitometry. The next three chapters deal with instrumental requirements, design, performance, operating characteristics and with methods and materials, concluding with a brief survey of commercial instruments.

The remainder, and much larger part, of the book is divided into 28 chapters contributed by different authors who between them cover many applications of TLC - densitometry in the pharmaceutical, biochemical and biomedical fields. The topics range, alphabetically, from alkaloids to vitamin K, but a few stand apart in subject or content. The chapter on planimetry of bioautograms is unexpected, but is included, as the editors explain, because of the absence of a known scanning method and the importance of the subject. Other chapters bordering the main theme deal with inorganic materials, colorants in food and drugs and with pesticides.

The contributing authors have mostly worked to a common format; each subject chapter consists of a brief introduction to the subject, a survey of the analytical background, not necessarily confined to densitometry, and a detailed description of laboratory work, often of a research nature, related to the author's particular field of interest. Most chapters conclude with a discussion of the work and all have references. There is a little repetition of basic principles, but otherwise the editors have largely succeeded in co-ordinating the writings of the 49 authors.

A few typographical errors are to be found, Figs. 3.5 and 3.6 appear to have been transposed and the typescript presentation, whatever its production merits, suggests, probably unintentionally, the transitory nature of most publications in steadily developing fields. Nevertheless, those already committed to TLC - densitometry will find the variety of approaches adopted by individual authors stimulating and worthwhile reading, even if their own specialist subject is absent. Anyone seriously considering the purchase of a commercial densitometer for TLC and uncertain of the best choice of instrument, or what to look for as regards cost/performance and versatility, should benefit greatly by first studying the relevant sections of this book. W. H. C. SHAW

CARBON-13 NMR IN POLYMER SCIENCE. BASED ON A SYMPOSIUM SPONSORED BY THE MACROMOLECULAR SCIENCE DIVISION AT THE 61ST CONFERENCE OF THE CHEMICAL INSTITUTE OF CANADA IN WINNIPEG, MANITOBA, JUNE 4–7, 1979. Edited by WALLACE M. PASIKA. *ACS Symposium Series* 103. Pp. x + 334. Washington, D.C.: American Chemical Society. 1979. Price $29.75.

A major aim of any worthwhile symposium is to bring to the attention of the delegates new and exciting developments in the particular field concerned. A considerable number of people interested in this area are, however, prevented from attending and it is to these people that this publication, covering the given papers and discussion, is directed, thus enabling a much wider audience to be aware of the advances recently made in the area of ^{13}C NMR spectroscopy in polymer science.

The delegates were treated to a series of 14 excellent papers covering a wide range of topics in the context of ^{13}C NMR spectroscopy of polymers. It is clearly not possible to comment on all the papers in a review of this nature so the following comments are intended to give the reader an impression of the comprehensive coverage given to the subject of the symposium. Thus, we are able to read of the prospects of dealing with solid polymeric materials both in a molecular structure analytical sense and in attempting to understand the molecular motion of the various ^{13}C functional groups present (A. N. Garroway *et al.*). In the biological science area the use of ^{13}C NMR spectroscopy is well demonstrated in the study of binding of hormones to receptor membranes (J. C. Jarrell *et al.*) making particular use of the dependence of relaxation times and chemical shifts on the pH of the systems under consideration. The very useful review type of paper on polymer stereochemical configuration (J. C. Randall) is a contribution worthy of note as it brings the stereochemical analysis of vinyl polymers clearly into focus and also provides the reader with a good list of references for further study. Another interesting paper (A. A. Jones *et al.*) concerns, more specifically than the majority, the use of various models to relate polymer motions to ^{13}C spin relaxation. This paper deals with amorphous bulk polymers and those which can be readily dissolved and clearly there are a number of somewhat ill-defined areas in this study but the potential of this sort of work in understanding chain dynamics is well indicated.

The visual presentation of these papers is good, in general, although some spectra are rather obviously hand copied from the original data (pp. 31 and 39). This is a practice that tends to detract from the content of the paper, especially if, in the same publication, excellent original spectra are also presented (pp. 126 and 297).

This publication in the *ACS Symposium Series* is a worthwhile addition to the bookshelf of any NMR spectroscopist actively concerned in the use of ^{13}C NMR spectroscopy to study polymeric molecules. It is good value at $29.75 and both the Editor, Wallace M. Pasika, and the publishers are to be congratulated on producing this volume in such a short space of time from the presentation of the papers at the symposium held in June 1979.

M. E. A. CUDBY

Divalent (Water Hardness) Ion-selective Electrodes Based on Poly(vinyl Chloride) and Poly(methyl Acrylate) Matrix Membranes

Poly(methyl acrylate) (PMA) provides an alternative support matrix to poly(vinyl chloride) but the usual 0.17 g of polymer established for functional poly(vinyl chloride) ion-selective electrodes must be increased to 0.3 g to produce master membranes of sufficient mechanical strength. The best membrane compositions were: PMA (0.3 g), calcium bis(didecylphosphate) (0.02 g), didecylphosphoric acid (0.02 g) with either 0.09 or 0.18 g of decan-1-ol. Electrodes with such sensor membranes showed near-Nerstian response to calcium and magnesium and functioned for about 10 days. Selectivity coefficients for divalent character with respect to calcium and magnesium measured by separate and mixed solution techniques were close to unity in many instances. The useful pH range lay between approximately 2.7 and approximately 7.2 in solutions of either 10^{-1} M calcium chloride or 10^{-1} M magnesium chloride levels and at their 10^{-3} M levels.

Neither the PMA electrode nor two others based on PVC matrix membranes with divalent sensor systems permitted accurate assays of total calcium and magnesium in standard analates. However, a PVC calcium ion-selective electrode based on calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]-phosphate} and dioctyl phenylphosphonate facilitated the accurate assay of calcium in the presence of three times its amount of magnesium.

Keywords: *Ion-selective electrode with poly(methyl acrylate) matrix membrane*; *divalent ion-selective electrode*; *water hardness electrode*; *polymeric membranes*; *alkyl phosphate sensors*

S. K. A. G. HASSAN, G. J. MOODY and J. D. R. THOMAS

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Analyst, 1980, **105**, 147–153.

Biamperometric Determination of Ethanol, Lactate and Glycerol Using Immobilised Enzymes in Flow Streams

Alcohol dehydrogenase was successfully immobilised on a nylon tube and on silanised glass beads packed in a short column, by means of covalent bonding. Lactic dehydrogenase and glycerol dehydrogenase were immobilised on titanium dioxide beads by adsorption, and on silanised glass beads by covalent bonding. All immobilisations by covalent bonding were achieved by means of glutaraldehyde cross-linking. These enzyme reactors were used in a flow system to measure ethanol, lactate and glycerol by catalysing their reaction with NAD. The NADH produced was coupled with hexacyanoferrate(III) ion, using diaphorase as a catalyst. The hexacyanoferrate(II) ion produced was measured biamperometrically using two open-tubular carbon electrodes. Measurement of standards in serum control samples was demonstrated.

Keywords: *Immobilised enzymes*; *NADH*; *hexacyanoferrate(II)*; *biamperometry*; *serum*

ABDULRAHAMAN S. ATTIYAT and GARY D. CHRISTIAN

Department of Chemistry, University of Washington, Seattle, Wash. 98195, USA.

Analyst, 1980, **105**, 154–160.

SITUATIONS VACANT

Determination of Ronidazole in Animal Feeds by Gas - Liquid Chromatography: A Collaborative Study by the EEC Committee of Experts

Report prepared by the Medicinal Additives in Animal Feeds Sub-Committee "A"

A previously published gas - liquid chromatographic procedure with flame-ionisation detection for the determination of ronidazole in feeding stuffs has been investigated by inter-laboratory studies and with minor modifications has been recommended as a method for use by the EEC. Collaborative studies have shown the method to be acceptable for enforcement purposes. The ronidazole is extracted from the feeding stuff with ethyl acetate and the solution is cleaned up by transferring the ronidazole, as its hydrochloride, into an aqueous solution, liberating the ronidazole by making the solution alkaline and re-extracting with ethyl acetate. The solvent is then evaporated, the ronidazole converted into a volatile silyl derivative by reaction with *NO*-bis(trimethylsilyl)acetamide in hot quinoline solution and the reaction mixture, with the addition of diethyl phthalate as internal standard, is then examined by gas - liquid chromatography.

Keywords: *Ronidazole determination*; *animal feeds*; *coccidiostats*; *gas - liquid chromatography*; *silylation*

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The Chemical Society, Burlington House, London, W1V 0BN.

Analyst, 1980, **105**, 161–164.

Study of Certain 1,5-Benzodiazepine Derivatives as Acid - Base Indicators

Short Paper

Keywords: *1,5-Benzodiazepine derivatives*; *acid - base indicators*; *dissociation constants*; *potentiometric measurement*

NAWAL A. EL-RABBAT, ADEL F. YOUSSEF and NABIL M. OMAR
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Analyst, 1980, **105**, 165–169.

Spectrophotometric Determination of Oxyphenbutazone in Pharmaceutical Preparations

Short Paper

Keywords: *Oxyphenbutazone determination*; *spectrophotometry*; *pharmaceutical preparations*

A. ABOU OUF, M. I. WALASH, S. M. HASSAN and S. M. EL-SAYED
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

Analyst, 1980, **105**, 169–173.

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Method for the Determination of Phosphorus in Lipids and Lipid-containing Materials

Short Paper

Keywords: *Phosphorus determination*; *phospholipids*; *alkali digestion*; *PTFE crucible*; *spectrophotometry*

L. HARTMAN, M. CARDOSO ELIAS and W. ESTEVES
Faculty of Food and Agricultural Engineering, State University of Campinas S.P., Brazil.

Analyst, 1980, **105**, 173–176.

Gravimetric Determination of Zinc by Precipitation with Benzimidazole Directly and from Homogeneous Solution by Replacement of Zinc in a Complex with EDTA

Short Paper

Keywords: *Zinc determination*; *gravimetry*; *precipitation with benzimidazole*; *zinc - EDTA complex*; *calcium replacement of zinc*

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Analyst, 1980, **105**, 176–179.

Separation and Identification of Mixtures of Urea and Hydroxymethyl-substituted Ureas by Paper Chromatography

Short Paper

Keywords: *Urea and hydroxymethylurea separation*; *paper chromatography*

R. TAYLOR, R. J. PRAGNELL and J. V. McLAREN
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Analyst, 1980, **105**, 179–181.

Improved Method for Detecting Microgram Amounts of Iron

Short Paper

Keywords: *Iron test*; *iron catalysis*

A. J. PANDELL, R. A. MONTGOMERY and R. A. MEISSNER
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Analyst, 1980, **105**, 181–183.

Application of Transition-metal Salts as Ion-pair Reagents in the Liquid Chromatography of Dithiocarbamates

Communication

Keywords: *Dithiocarbamate analysis*; *liquid chromatography*; *ion-pair reagents*; *transition-metal salts*

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Analyst, 1980, **105**, 184–185.

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ANALAO 105 (1247) 97–192 (1980) February 1980
ISSN 0003–2654

THE ANALYST

THE ANALYTICAL JOURNAL OF THE CHEMICAL SOCIETY

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Printed by Heffers Printers Ltd Cambridge England
Entered as Second Class at New York, USA, Post Office