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# Summaries of Papers in this Issue

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Careful selection of the conditions under which the steel samples are dissolved makes it possible to obtain final solutions with approximately the same concentrations with respect to nitric acid. In consequence, it is possible to analyse all of the steels examined by using the same calibration graph. The proposed method is free of interferences from the other components normally present in steels and is suitable for analysing samples with a tin content in the range 0.002–0.040%.

The proposed method provides rapid and accurate results and is suitable for routine analysis.

*Keywords*: *Tin determination*; *steels*; *atomic-absorption spectrophotometry*; *electrothermal atomisation*

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*Analyst*, 1977, **102**, 489–494.

## Direct Determination of Cadmium and Zinc in Sea Water by Carbon Furnace Atomic-absorption Spectrometry

A simple and rapid method is described for the determination of cadmium and zinc in sea water, using atomic-absorption spectrometry with carbon furnace atomisation. Samples, diluted 1+1 with de-ionised water, are injected into the carbon furnace and atomised in an HGA-72 furnace atomiser under gas-stop conditions. A low atomisation temperature of 1492 °C is used to separate the atomic-absorption signals from background absorption. Detection limits ($2\sigma$) of 0.04 $\mu$g $l^{-1}$ for cadmium and 1.7 $\mu$g $l^{-1}$ for zinc are reported. These limits appear to be adequate for all but the cleanest sea water samples. The use of standard addition is essential because of the interference from magnesium chloride and also when samples of varying salinity have to be analysed.

*Keywords*: *Cadmium determination*; *zinc determination*; *sea water*; *atomic-absorption spectrometry*

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*Analyst*, 1977, **102**, 495–502.

## Rapid Determination of Sulphate in Natural Waters and Plant Digests by Continuous Flow Injection Turbidimetry

The turbidimetric determination of sulphate as barium sulphate has been adapted to the continuous flow injection procedure of Růžička and co-workers. The effects of reagent composition, reagent flow-rates and length of mixing coils have been investigated. Optimum conditions were established for the determination of sulphate in natural waters and in plant digests. The results agree satisfactorily with those obtained by a standard turbidimetric method and analyses can be carried out at a rate of up to 180 samples per hour.

*Keywords*: *Sulphate determination*; *water*; *plant material*; *turbidimetry*; *flow injection analysis*

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*Analyst*, 1977, **102**, 503–508.

## Solid-state Ion-selective Electrodes for Metal Ions

Ion-selective electrodes for divalent metal ions ($Cu^{2+}$, $Pb^{2+}$, $Cd^{2+}$, $Hg^{2+}$) have been prepared by covering ionic conductors such as $Ag_2S$, $Ag_3SBr$ or $Ag_3SI$ with a thin metal sulphide layer. The metal sulphide layer was treated by heating under a partial pressure of sulphur. The observed standard potential is a good criterion for judging electrode performance.

*Keywords*: *Ion-selective electrodes*; *metal sulphide layers*; *divalent metal ions*

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*Analyst*, 1977, **102**, 509–514.

## Comparison of Two Different Types of Sodium Standards for Electron-probe Analysis of Soft Tissue

Standards prepared from sodium-bearing plastic were examined with an electron probe and compared with standards prepared from freeze-dried, plastic-embedded 20% albumin solution. The calibration coefficients for sodium were $3.062 \pm 0.038$ mequiv $l^{-1}$ $count^{-1}$ $s^{-1}$ with plastic standards and $2.66 \pm 0.11$ mequiv $l^{-1}$ $count^{-1}$ $s^{-1}$ with albumin standards. The accelerating voltage was 10 kV and counting rates were normalised to $7605 \pm 29$ count $s^{-1}$ for sodium chloride crystals. The difference in calibration factors is compatible with independent measurements, which indicate shrinkage of the albumin standards as a result of freeze drying.

*Keywords*: *Sodium standard comparison*; *electron-probe analysis*; *soft tissue analysis*

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The Analyst

# Determination of Tin in Plain Carbon, Stainless and High-speed Steels by Atomic-absorption Spectrophotometry with Electrothermal Atomisation

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A method for determining the tin content of plain carbon, stainless and high-speed steels by use of an atomic-absorption technique with electrothermal atomisation is described.

Careful selection of the conditions under which the steel samples are dissolved makes it possible to obtain final solutions with approximately the same concentrations with respect to nitric acid. In consequence, it is possible to analyse all of the steels examined by using the same calibration graph. The proposed method is free of interferences from the other components normally present in steels and is suitable for analysing samples with a tin content in the range 0.002–0.040%.

The proposed method provides rapid and accurate results and is suitable for routine analysis.

*Keywords*: *Tin determination*; *steels*; *atomic-absorption spectrophotometry*; *electrothermal atomisation*

Most methods used for determining the tin content of high- and low-alloy steels are based on polarographic[1] or spectrophotometric techniques.[2–5] With either technique the methods are time consuming and difficult to carry out as a result of the complicated separations required in order to eliminate the interfering elements. Classical atomic-absorption spectrophotometry, owing to its very high selectivity and great rapidity, eliminates most of these difficulties but it can be applied to tin determinations only when the tin content of the sample solution is greater than 2.4 $\mu$g ml$^{-1}$. In fact, if a dinitrogen oxide - acetylene flame is used (necessary in order to attain a sufficiently high temperature to dissociate the oxides), the sensitivity for tin is only 2.4 $\mu$g ml$^{-1}$ for 1% absorption. Several workers have carried out tin determinations in steels by using atomic-absorption spectrophotometry, but because of the aforementioned problems concerning the direct determination of tin[6] they have had to apply the conventional extraction technique[7–9] or devices that are either sophisticated[10,11] or incapable of detecting tin at levels below 0.005%.[12]

Sensitivities far higher than those typically obtained with classical atomic-absorption spectrophotometry were obtained by Ratcliffe *et al.*[13] with an electrothermal atomisation system using a graphite furnace. This method can be applied only to plain carbon steels.

In this paper, a method for the determination of tin in all kinds of steels based on the adoption of the electrothermal atomisation system is described. The combination of the high temperatures that can be attained with a graphite furnace, the carefully selected materials used in its construction and the possibility of operating with an atmosphere of inert gas have made it possible to develop a technique that is more time saving than any employed up to now and which is, therefore, particularly well suited to routine analysis.

## Experimental

### Apparatus

Experimental results were obtained by use of a Perkin-Elmer, Model 403, atomic-absorption spectrophotometer equipped with an HGA–74 electrothermal atomisation system, a hollow-cathode lamp for tin and a Perkin-Elmer, Model 165, recorder. The graphite furnace was operated with a controlled atmosphere of argon at 17.6 lb in$^{-2}$.

## Reagents and Solutions

*Orthophosphoric acid, sp. gr.* 1.71.

*Nitric acid, sp. gr.* 1.42.

*Hydrochloric acid, sp. gr.* 1.19.

*Standard tin solution,* 1 000 $\mu$g ml$^{-1}$. Dissolve 1 g of tin with hydrochloric acid in a 250-ml beaker. Heat the solution and then allow it to cool completely. Place it in a 1 000-ml calibrated flask and dilute it with nitric acid (20% *V*/*V*).

*Diluted standard tin solution,* 10 $\mu$g ml$^{-1}$. Dilute 10 ml of the standard tin solution to 1 000 ml with nitric acid (20% *V*/*V*).

*Iron.* Pure.

## Method

*Plain carbon and low-alloy steels*

Place 0.5 g of very fine drillings of the steel in a 250-ml beaker. Add 40 ml of diluted nitric acid (1 + 1) and heat the mixture until the steel sample has completely dissolved. Cool, and make the solution up to volume with water in a 100-ml calibrated flask. Remove 10 $\mu$l of the solution with an Eppendorf micropipette and place it in the graphite furnace. Carry out the analysis according to the working conditions listed in Table I.

TABLE I

WORKING CONDITIONS

| | Time/s | Selector position | Temperature/ °C |
|---|---|---|---|
| Drying | 20 | 70 | 100 |
| Thermal decomposition | 30 | 415 | 1 400 |
| Atomisation | 15 | 990 | 2 660 |
| Heating out | 5 | 999 | 2 700 |

| | | | |
|---|---|---|---|
| Wavelength | 224.6 nm | Range | Ultraviolet |
| Slit | 0.7 nm | Full-scale deflection | 1 A |
| Heating current | 30 mA | Recorder range | 10 mV |
| Background correction | Not used | Chart speed | 20 mm min$^{-1}$ |
| Gas flow-rate | Miniflow | | |

*Calibration graph.* Place 0.5 g of pure iron in each of a series of six 250-ml beakers. Add 40 ml of diluted nitric acid (1 + 1) to each and heat until the iron is completely dissolved. Allow the solutions to cool, then to each of the six solutions obtained add one of the six standard tin solutions listed in Table II.

TABLE II

VOLUMES AND TIN CONTENTS OF STANDARD TIN SOLUTIONS ADDED TO PURE IRON SOLUTION

| Diluted standard tin solution/ml | Amount of tin/$\mu$g | Tin concentration, % |
|---|---|---|
| 1 | 10 | 0.002 |
| 2 | 20 | 0.004 |
| 5 | 50 | 0.010 |
| 7.5 | 75 | 0.015 |
| 12.5 | 125 | 0.025 |
| 17.5 | 175 | 0.035 |

Transfer each solution into a 100-ml calibrated flask. Make the solutions up to volume with water and analyse 10 $\mu$l of each solution with the spectrophotometer according to the experimental conditions listed in Table I.

*Stainless steels*

Place 0.5 g of very fine drillings of the stainless steel in a 250-ml beaker and dissolve it with 10 ml of concentrated hydrochloric acid and 15 ml of concentrated nitric acid. Heat the mixture until the steel has completely dissolved and evaporate the resulting solution until the volume is approximately 5 ml. Add 10 ml of concentrated nitric acid and make the solution up to volume with water in a 100-ml calibrated flask. Remove a 10-$\mu$l sample and determine its tin content by using the spectrophotometer with the working conditions listed in Table I.

The calibration graph determined for plain carbon steels can also be used for stainless steels.

*High-speed and extra-high-speed steels*

Place 0.5 g of very fine drillings of the steel in a 250-ml beaker and dissolve it with 5 ml of orthophosphoric acid, 5 ml of concentrated hydrochloric acid and 15 ml of concentrated nitric acid. Heat the mixture until the steel has completely dissolved and allow the solution to cool, then make it up to volume with water in a 100-ml calibrated flask. Remove a 10-$\mu$l sample and determine its tin content by using the spectrophotometer with the experimental conditions listed in Table I. The calibration graph determined for plain carbon steels can also be used for these steels.

## Discussion

### Basic Considerations

Preliminary experimental tests proved that slightly acidic (acidified with nitric acid) tin solutions with a tin content as low as 0.02 $\mu$g ml$^{-1}$ can be analysed successfully using the atomic-absorption method with electrothermal atomisation. This value is well beyond the sensitivity attainable with classical atomic-absorption techniques, which require a tin content of at least 2.4 $\mu$g ml$^{-1}$ in order to obtain an absorption of 1%.

The greater sensitivity to tin allowed by using atomic-absorption spectrophotometry with electrothermal atomisation made it possible to consider the problem of determining the low content of tin in steels by a direct method. However, in order to apply successfully the direct technique, several concomitant problems, some of a chemical nature (steel sample size, matrix type, sample attack, final solution acidity, interference phenomena, etc.), others of a technical nature (influence of gas flow-rate, influence of temperature and time required to carry out specific thermal treatments, etc.) had to be overcome.

### Atomisation with Gas Stop and with Miniflow

The atomisation of elements by use of the atomic absorption with electrothermal atomisation technique can be carried out in the presence or absence of an inert gas. In the latter instance (gas stop), the atoms in the graphite tube remain in the path of the beam for far longer periods of time (approximately 8 s), thereby increasing the sensitivity to a considerable degree.

The increase in sensitivity obtained in this manner may reach values that lead to a non-linear response; in some instances, therefore, it is preferable to work with a reduced gas flow. For this reason, and also because the test procedure is simpler, it was decided to operate under Miniflow conditions; it was then possible to determine a far wider range of tin concentrations without having to adjust test parameters such as mass of sample, dilution ratio and final amount of sample.

### Sample Mass (Influence of Iron)

Iron is the main constituent of any steel matrix; it is also an element that is far more difficult to atomise than tin, so that atomisation of tin during spectroscopic analysis always takes place in the presence of massive amounts of iron. Tests were carried out to ascertain whether, and to what extent, tin absorption peaks were influenced by the iron present.

The influence of iron on tin absorption peaks appears to be negligible (at least for the range of iron and tin contents considered, *viz.*, 0–2 g of iron per 100 ml and 0.4 $\mu$g ml$^{-1}$ of tin) in that the intensification effects obtained can be attributed to amounts of tin present in the iron as impurities. Nevertheless, as a precautionary measure, it was decided to adopt a value of 0.5 g per 100 ml for the ratio between the mass of the steel sample and solution volume and to determine the calibration graph in the presence of pure iron.

### Amount of Solution to be Atomised

The graphite furnace can accept amounts of solution varying from a minimum of 1 $\mu$l to a maximum of 100 $\mu$l. Unless specifically indicated to the contrary, the analyses were always carried out on 10 $\mu$l of solution. It was noted that the reproducibility of the measure-

ment was impaired when the amount of solution was increased or when a diminished amount was used; in the latter instance the cause of the impairment could have been the excessively small amount of the sample combined with the difficulty of drawing exactly the same volume with the micropipette on each occasion.

## Thermal Decomposition and Atomisation: Temperature and Time Intervals

In the classical atomic-absorption method the sample passes directly from the liquid to the atomised state. With the graphite furnace this transformation takes place gradually and it is possible to submit the sample (if necessary or desirable) to heat treatments during the process. In our work the sample first underwent thermal decomposition and then it was atomised.

This treatment makes it possible to eliminate the solvent and to modify the molecular structure of the species under examination. The following optimum time and temperature values were established experimentally for determining the tin content of a steel sample dissolved in 20% *V*/*V* nitric acid: for thermal decomposition, 30 s at 1400 °C; and for atomisation, 15 s at 2 660 °C.

Nitric acid was preferred to hydrochloric acid as the solvent because nitrate salts are less volatile than chloride salts. An acidity of 20% *V*/*V* was chosen for the nitric acid as the acid dissolves plain carbon steel at this concentration. The additional reagents required for the complete dissolution of the other types of steel examined were selected with a view to keeping this particular parameter unaltered, so as to obtain the same final conditions for all of the solutions analysed.

## Optimum Sample Dissolution Conditions

Plain carbon steels can be dissolved completely with nitric acid alone (20% *V*/*V*), but alloy and high-speed steels require the presence of other acids as well. For example, a stainless steel is merely passivated when it is attacked with nitric acid only; high-speed steels contain tungsten, which has to be complexed with orthophosphoric acid before the steel can be dissolved completely by the nitric acid.

The different behaviour of the various steel matrices under nitric acid attack gives rise to different final tin solutions and involves the production of a specific calibration graph for each type of steel analysed.

A study was therefore made to find reagents which, added to the nitric acid, would produce final solutions of the different kinds of steel on which the tin determination could be carried out by using one common calibration graph. For this purpose, stainless steel was attacked with aqua regia and, because it was feared that the chlorides would volatilise during the thermal decomposition stage, the hydrochloric acid was eliminated with sulphuric acid, evaporating the solution until fumes of sulphur dioxide appeared; the residue was subsequently dissolved in nitric acid (5% *V*/*V*). The same procedure was followed for high-speed steels following the addition of orthophosphoric acid. In both instances, however, it was noted that the sulphuric acid present in the solution decreased the tin absorption peaks excessively.

As a consequence of this interference, sulphuric acid was rejected as a reagent and the stainless steel was dissolved with 10 ml of hydrochloric acid and 15 ml of nitric acid. The hydrochloric acid was eliminated by evaporating the solution down to 5 ml. Conditions identical with those in the plain carbon steel solution were obtained by adding 10 ml of concentrated nitric acid before diluting to volume with water. A similar procedure was evolved for high-speed steels, which were dissolved with 5 ml of hydrochloric acid, 15 ml of nitric acid and 5 ml of orthophosphoric acid.

Fig. 1 shows the calibration graph obtained by analysing synthetic solutions, the compositions of which correspond to plain carbon, stainless and high-speed steels. As the graph is valid for all kinds of steels examined, it can be assumed that chromium, nickel and tungsten have little or no influence on the determination of tin. It can be seen that the graph does not pass through the origin. If, however, a deuterium background corrector is used, a similar graph is obtained that does pass through the origin.

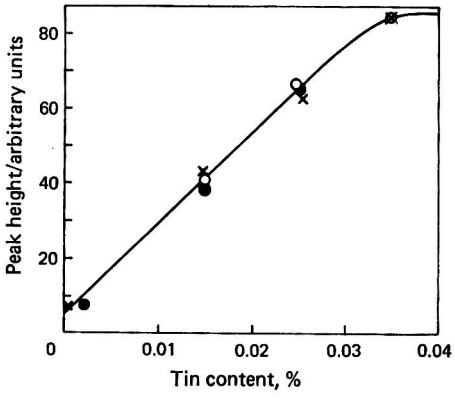


Fig. 1. Calibration graph for tin content of steel samples. ●, Plain carbon steel; ○, stainless steel; and ×, high-speed steel.

## Results

The proposed method was checked on a series of steel standards with a wide range of compositions (BCS, NBS, IRSID, JSS and BAM). The values obtained by use of the adopted procedure compared with the certified tin contents are given in Table III.

TABLE III

RESULTS FOR DETERMINATION OF TIN

| Sample | Certified tin content, % | Tin content found, % | Difference |
|---|---|---|---|
| NBS 51B .. | 0.008 | 0.009 | +0.001 |
| NBS 65d .. | 0.004 | 0.004 | — |
| JSS 112–1 .. | 0.002 | 0.002 | — |
| JSS 156–1 .. | 0.026 | 0.024 | −0.002 |
| JSS 157–1 .. | 0.017 | 0.014 | −0.003 |
| NBS 101e .. | 0.020 | 0.020 | — |
| BCS 431 .. | 0.006 | 0.006 | — |
| BCS 432 .. .. | 0.016 | 0.016 | — |
| BCS 434 .. .. | 0.011 | 0.011 | — |
| BCS 239/3 .. | 0.030 | 0.025 | −0.005 |
| BCS 218/3 .. | 0.042 | 0.042 | — |
| BAM Sthal 14 .. | 0.020 | 0.021 | +0.001 |
| 2Cr Ni Mo .. | (0.006) | 0.007 | +0.001 |
| NBS 50c .. | 0.018 | 0.016 | −0.002 |
| BCS 241/2 .. | 0.025 | 0.027 | +0.002 |

It can be seen that the experimental results are in good agreement with the certified values. Reproducibility of the measurement procedure was established by taking nine consecutive readings, expressed as height of peak in chart divisions, on 10-$\mu$l volumes of a 0.5 $\mu$g ml$^{-1}$ solution of tin. The standard deviation was 1.51, the relative standard deviation being 5.4%. The reproducibility of the over-all method was established by repeating the analysis of standard sample BCS 434 (tin content 0.011%) 15 times. The results led to a standard deviation of 0.001% with a relative standard deviation of 9.43%.

## Conclusions

The proposed method, applied according to the conditions and procedures described in this paper, is valid for determining tin contents in the range 0.002–0.040% in plain carbon, stainless and high-speed steels. The standard deviation of the method is 0.001%.

The time required for performing the complete analysis (including the time required for dissolving and atomising the sample) is 10 min; the method is therefore particularly suited to routine analyses. It is exempt from interferences, at least as far as normal concentrations of the elements usually present in steels are concerned.

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# Direct Determination of Cadmium and Zinc in Sea Water by Carbon Furnace Atomic-absorption Spectrometry

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A simple and rapid method is described for the determination of cadmium and zinc in sea water, using atomic-absorption spectrometry with carbon furnace atomisation. Samples, diluted 1+1 with de-ionised water, are injected into the carbon furnace and atomised in an HGA-72 furnace atomiser under gas-stop conditions. A low atomisation temperature of 1492 °C is used to separate the atomic-absorption signals from background absorption. Detection limits ($2\sigma$) of 0.04 $\mu$g $l^{-1}$ for cadmium and 1.7 $\mu$g $l^{-1}$ for zinc are reported. These limits appear to be adequate for all but the cleanest sea water samples. The use of standard addition is essential because of the interference from magnesium chloride and also when samples of varying salinity have to be analysed.

*Keywords*: *Cadmium determination*; *zinc determination*; *sea water*; *atomic-absorption spectrometry*

The concentrations of toxic trace elements in sea water are of considerable interest at present.[1] Analytical techniques are required that can be used to monitor the levels of such elements in both coastal and oceanic waters, so that data on the differences between locations and in time can be assessed. Metals such as cadmium, lead and zinc accumulate in the oceans from sources such as atmospheric fallout, rivers and direct dumping of waste into the sea. Many types of marine life, including shellfish, fish, plankton and birds, are known to concentrate these elements[1–3] which, besides posing an increasing threat to marine life, may also affect man. The average levels of these three elements in sea waters are in the micrograms per litre concentration range and values reported over the world's oceans are compared with those for the River Clyde area in Table I.

TABLE I

REPORTED CONCENTRATIONS OF CADMIUM, LEAD AND ZINC IN SEA WATER

| Element | Oceanic concentrations[4]/ $\mu$g $l^{-1}$ | Clyde sea concentrations[3]/ $\mu$g $l^{-1}$ |
|---|---|---|
| Cadmium | 0.03–0.17 | 0.01–0.24 |
| Lead | 0.03–9.0 | 0.02–0.36 |
| Zinc | 0.3–10.0 | 2.0–23.0 |

The direct determination of these elements at these concentrations in a sea water matrix is extremely difficult because of the lack of sensitivity of most techniques, the problem of contamination during collection and transfer of the samples to the laboratory and the interference of the matrix itself.[1]

Atomic-absorption spectrometry is one of the most suitable techniques for the analysis of trace metals in natural waters, including sea water, and this subject has recently been reviewed.[1,5] The aspiration of neat sea water into a flame generally results in scatter of radiation and clogging of the burner by the high salt concentration, and the separation and pre-concentration of trace elements in sea water is necessary in order to achieve analysis by flame-atomisation atomic-absorption spectrometry.[1,5] The use of separation and pre-concentration techniques will obviously lead to the possibility of error from additional sources of contamin-

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ation unless considerable care is taken, and a simple, direct, more rapid method would be desirable. If the accuracy of such a method could be validated, the possible sources of contamination would be reduced and an increased throughput of samples achieved. Burrell[1] recently expressed the hope that the new atomisation techniques in atomic-absorption spectrometry would provide such methods.

In describing the Perkin-Elmer HGA-70 carbon furnace atomiser, Fernandez and Manning[6] reported sensitivities of 0.03 $\mu$g l$^{-1}$ for cadmium, 0.76 $\mu$g l$^{-1}$ for lead and 0.04 $\mu$g l$^{-1}$ for zinc, which suggest the possibility of determining cadmium and zinc in sea water directly; this appears to be impossible with lead. However, Segar and Gonzalez[7] showed that the high salt content of sea water gave rise to a large scatter signal during carbon furnace atomisation and found that this could not be compensated for by the background correction system used. This technique was used for the direct determination of natural levels of iron in sea water and by selective volatilisation the determination of copper, manganese, cobalt, nickel and vanadium in polluted sea water was also achieved. Direct determination of cadmium, lead and zinc was found to be impossible and the use of separation procedures with carbon furnace atomic-absorption methods has been proposed by several groups of workers.[8–10]

When a sample of sea water is atomised in a carbon furnace a large non-atomic absorption signal is obtained at many wavelengths, including the resonance wavelengths for cadmium, lead and zinc. This is mainly a result of molecular absorption by molecules such as sodium and calcium chlorides, but in some designs of furnace it can also be caused by scattering of radiation by particles formed on condensation of the matrix vapour with the atmosphere. Background correction techniques involving the use of a deuterium arc or hydrogen lamp can be used to overcome this absorption, but if the background signal is sufficiently intense, accurate compensation is impossible and the net response is no longer that due only to the atomic-absorption signal. While the work described in this paper was in progress, Lundgren and co-workers[11–13] pointed out that by atomisation at a low temperature, the atomic signals of volatile elements can be differentiated in time from the non-atomic signals, and methods for the determination of cadmium in sodium chloride at sea water concentrations[12] and in blood[13] have been reported. Lundgren and co-workers[11–13] used a specially designed graphite furnace in which an infrared detector is used to control accurately the temperature of atomisation.

In the method described in this paper, an unmodified commercial instrument has been used to carry out the direct determination of cadmium and zinc in sea water samples. A low atomisation temperature is used in order to achieve separation in time of atomic and non-atomic absorption signals in an analogous manner to that of Lundgren and co-workers.[11–13] In addition the interferences of the main constituents of sea water have been investigated and it is shown that there is a depressive interference from magnesium chloride that reduces the sensitivity of the carbon furnace atomic-absorption signal. Despite this interference, the carbon furnace procedure described allows the determination of cadmium and zinc in sea water by a standard addition method following 1 : 1 dilution of the samples with de-ionised water.

## Experimental

### Reagents

Reagents of the highest available purity were used throughout and de-ionised water was used in the preparation of all solutions. Stock solutions of cadmium and zinc containing 2 000 $\mu$g ml$^{-1}$ of these elements were prepared from cadmium sulphate and zinc sulphate, respectively. They were diluted to 10 $\mu$g ml$^{-1}$ for a working stock solution as required and diluted further immediately before use. Contamination from glassware, stoppers and pipette tips was observed. All of the glassware was thoroughly cleaned with 2% *V*/*V* nitric acid and rinsed with de-ionised water used straight from the de-ioniser. Dilutions were also prepared with water directly from the column and kept for the minimum time before use.

### Apparatus

The instrument used for carbon furnace measurements was a Perkin-Elmer HGA-72 heated graphite atomiser, which was mounted in a Perkin-Elmer 306 atomic-absorption spectrometer equipped with a deuterium arc background corrector and an Electronic 194 strip-chart recorder. Perkin-Elmer Intensitron hollow-cathode lamps were used as sources. The design

and operation of the HGA-72 are similar to those of the HGA-70 and have been described in detail elsewhere.[6,14] Samples were transferred to the centre of the carbon tube by means of a 50-$\mu$l Eppendorf micropipette. The argon purge gas flow-rate was 1.5 l min$^{-1}$ at 40 lb in$^{-2}$.

In order to check the accuracy of the carbon furnace atomic-absorption procedures, comparative results for sea water samples were obtained by use of a flame atomisation atomic-fluorescence procedure. The apparatus used has been described elsewhere.[15,16] A Perkin-Elmer 290 atomic-absorption spectrometer was used in the emission mode and electrodeless discharge lamps, operated in a 210L Broida cavity and powered by a Microtron Mark I microwave generator, were used as light sources. The electrodeless discharge lamps were mechanically modulated, thermostatically controlled by a stream of heated nitrogen and mounted in the open space in front of the spectrometer. Solutions were nebulised into an air - hydrogen flame maintained on a circular burner head with two linear rows of nine holes.

## Procedures

### *Sample collection and storage*

Sea water samples were collected from the inner Clyde sea using a launch. Surface samples were collected by using a plastic bucket and samples from depth were collected using N.I.O. polypropylene water samplers, which were submerged to the required depth and closed by means of spring-loaded seals. Samples of 3- or 5-l volume were transferred rapidly into polyethylene sample bottles, with the addition of 1 ml of concentrated AnalaR nitric acid to stabilise the sea water with respect to cadmium, zinc and lead. The polyethylene bottles were cleaned by soaking them in 5% *V*/*V* nitric acid overnight, followed by thorough washing with de-ionised water.

### *Carbon furnace procedure*

Sea water samples are analysed by means of a standard addition procedure. All glassware must be thoroughly cleaned before use by soaking it in 5% *V*/*V* nitric acid overnight, followed by thorough washing with de-ionised water.

Pipette 25 ml of the sea water sample into a 50-ml calibrated flask and dilute to the mark with water from a de-ionisation column. Draw off and reject 50-$\mu$l aliquots of the solution with the micropipette at least three times. Then inject a 50-$\mu$l aliquot of this solution into the carbon furnace and atomise under the conditions given in Table II.

TABLE II

SAMPLE ATOMISATION CONDITIONS

| | Cadmium | Zinc |
|---|---|---|
| Wavelength/nm | 228.8 | 213.9 |
| Spectral band width/nm | 0.7 | 0.7 |
| Lamp current/mA | 10 | 18 |
| Drying temperature/°C | 100 | 100 |
| Drying time/s | 45 | 45 |
| Atomisation temperature/°C | 1 492 | 1 492 |
| Atomisation time/s | 10 | 10 |
| Volume of sample solution/$\mu$l | 50 | 50 |
| Scale expansion | ×3 | ×3 |
| Argon flow-rate/l min$^{-1}$ (at 40 lb in$^{-2}$) | 1.5 | 1.5 |

The gas-stop facility should be used during the atomisation stage. The deuterium arc background corrector should be used at all times and should be correctly aligned with the hollow-cathode lamp. The tube should be fired at its maximum temperature for 10 s after each sample to clear the sample matrix from the tube surface.

Prepare a calibration graph for each sample by the standard addition method by adding 20-$\mu$l aliquots of either 0.5 $\mu$g ml$^{-1}$ cadmium solution (equivalent to an addition of 0.2 $\mu$g l$^{-1}$) or 12.5 $\mu$g ml$^{-1}$ zinc solution (equivalent to 5 $\mu$g l$^{-1}$) to the 1 : 1 diluted sea water solution. At least two additions should be made to each sample. Obtain the cadmium or zinc concentrations from the standard addition calibration graph and multiply by 2 to obtain the concentration of each element in the sea water sample.

*Flame atomic-fluorescence procedure*

In order to check the accuracy of the carbon furnace procedure a batch of sea water samples was also analysed by flame atomic-fluorescence spectrometry, using an extraction - back-extraction procedure in order to separate the cadmium and zinc from the sea water matrix and to achieve a 100-fold concentration of these elements. All glassware had to be scrupulously cleaned before use by washing with 5% *V/V* nitric acid and de-ionised water.

One litre of sea water was placed in a 2-l beaker and 2 ml of 20% *V/V* AnalaR grade acetic acid were added. The pH was adjusted to 4.5–5 by addition of AnalaR ammonia solution and then the solution was transferred to a 5-l separating funnel fitted with a PTFE tap. A 10-ml volume of freshly prepared 1% ammonium tetramethylenedithiocarbamate (Koch-Light) and 30 ml of AnalaR chloroform were then added, and the contents of the separating funnel shaken vigorously for 5 min. When it had settled out, the chloroform layer was run into a 100-ml PTFE beaker. Following the addition of 2 ml of AnalaR nitric acid, the mixture was evaporated slowly to dryness on a low-temperature hot-plate. The residue was dissolved in 1 ml of AnalaR hydrochloric acid and 9 ml of de-ionised water were added.

The final solution was analysed by comparison with cadmium or zinc standards prepared in 10% *V/V* hydrochloric acid using the apparatus described above. Additions of cadmium and zinc to sea water samples were recovered with 100 ± 5% accuracy by use of this procedure.

## Results and Discussion

### Interference of Sea Water in Cadmium Determinations

The major constituents of sea water that are likely to affect the determination of trace elements such as cadmium and zinc are sodium, potassium, calcium and magnesium, all of which are present in a chloride medium at very high concentration. The effect of these elements on the carbon furnace atomic-absorption signal for 50 μl of a 0.003 μg ml$^{-1}$ solution of cadmium was examined at levels up to the approximate natural level of each element in sea water and the results are shown in Table III. All of the salts were added as their chlorides. In this analysis, the normal atomisation temperature for cadmium of 1 800 °C was used, but otherwise the conditions were as described under the carbon furnace procedure.

TABLE III

INTERFERENCE OF MAJOR SEA WATER ELEMENTS ON CADMIUM

Signals in chart divisions for 50 μl of 0.003 μg ml$^{-1}$ cadmium solution. For atomisation conditions, see Table II.

| Concentration/ μg ml$^{-1}$ | Calcium | Magnesium | Potassium | Sodium |
|---|---|---|---|---|
| 0 | 39 | 37 | 36 | 37 |
| 10 | 37 | 37 | 38 | 37 |
| 50 | 40 | 34 | 36 | 38 |
| 100 | 35 | 9 | 34 | 35 |
| 400 | 36* | 6 | 37* | 35 |
| 1 000 | — | 4 | — | † |
| 1 250 | — | 3* | — | † |
| 10 000 | — | — | — | †* |

* Indicates normal sea water concentration of each element.
† Background correction system failed.

It is clear, from Table III, that at the levels found in sea water, calcium and potassium have little effect on the signal given by this concentration of cadmium. However, at concentrations of 1 000 to 10 000 μg ml$^{-1}$, sodium interferes seriously as the background molecular absorption signal from sodium chloride is too great for the background correction system to overcome efficiently. At this atomisation temperature the cadmium and sodium chloride peaks are not differentiated in time during the atomisation stage. In separate experiments, additions of cadmium to a sample of sea water were made but no cadmium signal could be

observed in the presence of the intense background signal at concentrations of cadmium far in excess of those found in sea water.

It was also found that magnesium depressed the cadmium signal and this depression started at the 100 $\mu$g ml$^{-1}$ level, which is well below the level found in sea water. This depression appears to be due to a chemical interference between the cadmium and magnesium chloride. Similar effects of magnesium chloride have been observed on a number of elements.[17,18] In many instances[18] the effect of magnesium is removed by the introduction of an intermediate heating stage, to between 700 and 1 000 °C, before atomisation, but this is not possible for cadmium, lead or zinc as substantial amounts of these elements are vaporised during such treatment and the subsequent atomisation signals are low and of poor reproducibility.

Attempts were made to overcome the interferences of sodium and magnesium chlorides by using releasing agents. In earlier studies it had been shown that the presence of a relatively involatile matrix allowed reproducible atomisation of a volatile element, *viz.*, lead.[19,20] The presence of large concentrations of chloride ions makes the sea water matrix relatively volatile and it was hoped that the addition of 10000 $\mu$g ml$^{-1}$ of iron or calcium as the nitrate or sulphate would reduce the volatility of the matrix sufficiently to allow the absorption signals of the cadmium and matrix to be differentiated in time. These attempts were unsuccessful and the cadmium atomic-absorption signal could not be resolved from the non-specific background signal obtained during atomisation. Use of pre-atomisation heating was also unsuccessful as cadmium is lost by vaporisation above 350 °C and the bulk of the sea water matrix is not vaporised until much higher temperatures. The addition of ammonium nitrate has been recommended in order to overcome the effects of a chloride matrix[21] but this procedure could not be used for cadmium as insufficient ammonium chloride is driven off at the maximum ashing temperature possible for cadmium.

It was found that the addition of 1000 $\mu$g ml$^{-1}$ of aluminium and 1–4% *V*/*V* nitric acid did overcome the interference effect of magnesium and separated the atomic-absorption signal for cadmium from the background signal. Cadmium signals could then be observed with reasonable sensitivity. The addition of aluminium nitrate and nitric acid at the levels indicated caused a 50% depression of the cadmium signal, but addition of 1 250 $\mu$g ml$^{-1}$ of magnesium as the chloride, had no further effect on the signal. Unfortunately, when aluminium and nitric acid were added to sea water at these levels, and the sample was analysed by standard addition, a result of 2 $\mu$g l$^{-1}$ of cadmium was obtained. This was considerably in excess of values reported for the Clyde sea area[2] and it was thought to be due to the introduction of cadmium via the releasing agents. If the reagents can be purified sufficiently, this remains a suitable approach to this problem, particularly where the final procedure described here is unsuitable.

## Determination of Cadmium and Zinc in Sea Water

As the concentrations of these elements in sea water are very low, any method involving the addition of releasing agents is liable to introduce unacceptably high blank values. A method involving no additions was therefore sought and in order to achieve this it was necessary to obtain a separation of the signals given by cadmium or zinc from that of the sodium chloride, at least to an extent that would allow efficient operation of the background correction system.

With use of the optimum atomisation temperature for cadmium of 1 800 °C, attempts were made to separate atomic from background absorption signals by using a slower heating rate. The HGA-72 has a facility for controlling the rate of increase in temperature at ten pre-set values. This facility is normally used in the charring step, but it was thought that the cadmium atomic signal might appear well before that of the background if the tube temperature was raised more slowly. It was found that although the background signal was much reduced in magnitude through being extended over a much longer time, no cadmium signals could be observed from sea water samples before or after addition of cadmium to such samples. The instantaneous concentration of cadmium had been reduced to too low a level.

An alternative procedure was to raise the tube temperature to a value lower than the optimum at the normal rate of temperature increase during the atomisation stage. Careful selection of the temperature would then allow the measurement of cadmium at reduced sensitivity but before a substantial amount of background had been vaporised. This is analogous to the method proposed by Lundgren and co-workers.[11–13] In addition, the gas-stop facility on the HGA-72 was used to reduce the diffusion of cadmium out of the tube and to

build up its concentration in order to achieve an increase in sensitivity. The use of a fast rise to a low atomisation temperature allowed the cadmium to "drift off" in the absence of an excessive amount of background and the concentration of cadmium in the optical path was increased by using the gas-stop facility.

It was found that separation of cadmium from the background could be achieved at an atomisation temperature of 1492 °C (equivalent to 300 digits on the HGA-72) provided that the sea water sample was diluted at least 1:1 with de-ionised water. Cadmium atomic-absorption signals could then be measured in the presence of the sea water matrix. The temperature of 1492 °C gave the best sensitivity for the determination of cadmium, at which interference from the matrix was negligible. Although this technique overcame the interference from sodium it did not significantly alter the interference of magnesium, which is chemical in nature. It is therefore necessary to calibrate the instrument for sea water analysis using a sea water sample. One method of achieving this is to take a sample of sea water and remove the cadmium from it by extraction with ammonium tetramethylenedithiocarbamate - chloroform. Additions of cadmium can then be made to this sample in order to obtain a calibration graph. The recorder traces for such a graph are shown in Fig. 1 and indicate the linear response to cadmium additions in the 0.5–4.0 $\mu$g l$^{-1}$ concentration range; they also show that the cadmium atomic-absorption signal is obtained completely before breakdown of the background correction system occurs at X.

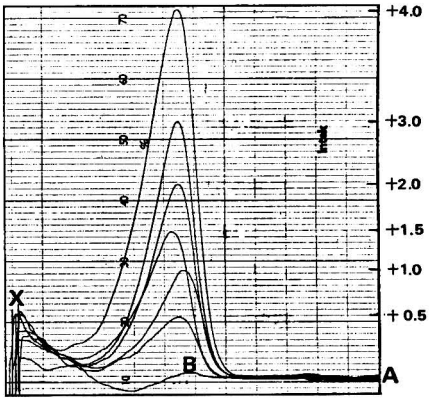


Fig. 1. Cadmium atomic-absorption signals obtained by adding 0.5–4.0 $\mu$g l$^{-1}$ of cadmium to a blank sea water (B) diluted 1:1 with de-ionised water. Atomisation of 50-$\mu$l aliquots at 1 492 °C. Recorder chart speed 1 cm s$^{-1}$. Atomisation starts at A. Background correction system breaks down at X.

Compared with the analysis of aqueous solutions of cadmium, loss of sensitivity is incurred as a result of three factors: the need to dilute the sample by 50%; the need to use a lower atomisation temperature; and the effect of depressive interference from magnesium chloride. A partial recovery of sensitivity is achieved by the use of the gas-stop facility. As the salinity of sea water samples was likely to vary considerably, particularly in the Clyde estuary, and the concentration of magnesium chloride would also vary, it was considered essential to analyse samples by standard addition to each sample rather than by use of a single calibration graph. At least two additions were made to each sample. Repetitive analysis of a single sample by separate dilution and standard addition yielded the results shown in Table IV. A detection limit, based on the concentration giving a signal equal to twice the standard deviation of this sample, of 0.04 $\mu$g l$^{-1}$ was obtained, which was sufficiently low for all of the samples taken from the Clyde sea area. The same procedure that was developed for cadmium was used to determine zinc and lead, and the results given in Table IV were obtained. With lead, no signal was observed from the 1:1 diluted sea water and a 125 $\mu$g l$^{-1}$ solution of lead had

to be added before measurable signals were obtained. The conditions for lead are not given in the experimental section but they were identical with those for cadmium, except that a lead hollow-cathode lamp was used at a wavelength of 283.3 nm and a lamp current of 10 mA.

TABLE IV

ANALYSIS OF SEA WATER FOR CADMIUM, LEAD AND ZINC USING HGA-72 CARBON FURNACE

| | Cadmium | Zinc | Lead |
|---|---|---|---|
| Mean value of sample/$\mu$g l$^{-1}$ | 0.10 | 10.9 | 125* |
| Number of separate determinations | 10 | 9 | 10 |
| Relative standard deviation | 20 | 7.8 | 8.0 |
| Detection limit/$\mu$g l$^{-1}$ (based on 2$\sigma$) | 0.04 | 1.7 | 40 |
| Sensitivity | 0.04 | 0.5 | 20 |

* 125 $\mu$g l$^{-1}$ of lead was added to the sample.

Comparison of the figures in Tables I and IV shows that the technique developed is suitable for the determination of cadmium and zinc in nearly all sea water samples, but is clearly unsuitable for the determination of lead in all but highly polluted waters. Attempts to improve the situation with respect to lead have not, so far, been successful. The detection limits quoted are based on twice the standard deviation of the analysis of a sample with a low concentration near to the detection limit. If a higher confidence level is required, the method may be considered unsuitable for even cadmium and zinc determinations at levels towards the lower ends of the ranges given in Table I. It would obviously be advantageous if the sensitivity and detection limit could be improved further for all three elements and it is to be hoped that improvements in furnace design may eventually make this possible. However, the present methods are suitable for many, but not all, sea water samples and will provide rapid screening procedures for the analysis of large numbers of survey samples. The results given in this paper identify the present possibilities for the direct determination of cadmium and zinc in sea water using carbon furnace atomisation. If additional sensitivity is required, it will still be necessary to resort to pre-concentration techniques before analysis.[8–10]

The accuracy of the procedure was assessed by comparative analysis of a batch of samples using the procedure described for atomic-fluorescence spectrometry with an air - hydrogen flame. Samples were collected and analysed within 2 d with both techniques. The samples were taken from the inner Clyde between Glasgow and Greenock, where the salinity was known to vary over a considerable range, and the results are shown in Table V. Reasonable agreement was found between the two procedures for both cadmium and zinc, confirming the accuracy of the carbon furnace procedure.

TABLE V

ANALYSIS OF CLYDE SEA WATER SAMPLES FOR CADMIUM AND ZINC BY CARBON FURNACE ATOMIC-ABSORPTION SPECTROMETRY (CFAAS) AND FLAME ATOMIC-FLUORESCENCE SPECTROMETRY (AFS)

| Sample number | Position of buoy/ miles from Glasgow | Salinity, % | Cadmium concentration/ $\mu$g l$^{-1}$ | | Zinc concentration/ $\mu$g l$^{-1}$ | |
|---|---|---|---|---|---|---|
| | | | CFAAS | AFS | CFAAS | AFS |
| 1 | 14 | 2.35 | 0.23 | 0.25 | 17.0 | 16.8 |
| 2 | 15 | 2.66 | 0.21 | 0.17 | 11.4 | 12.0 |
| 3 | 16 | 2.81 | 0.21 | 0.14 | 11.1 | 9.3 |
| 4 | 17 | 2.90 | 0.11 | 0.13 | 11.2 | 8.4 |
| 5 | 18 | 2.99 | 0.14 | 0.12 | 9.3 | 8.0 |
| 6 | 19 | 3.04 | 0.11 | 0.11 | 10.0 | 8.4 |

In their paper, Lundgren *et al.*[12] demonstrated the possibility of determining cadmium in the presence of 2% *m/V* sodium chloride and reported a detection limit for cadmium of 0.03 $\mu$g l$^{-1}$. They used an atomisation temperature of 820 °C in a specially designed

furnace, which had reduced, or possibly zero, gas flow and an atomisation temperature controlled by an infrared detector to $\pm 10$ °C. In this work, using an unmodified commercial instrument, the determination of cadmium and zinc in sea water samples has been demonstrated and a detection limit for cadmium of 0.04 $\mu g\ l^{-1}$ obtained. An atomisation temperature of 1 492 °C was used on the HGA-72, which is substantially higher than that recommended by Lundgren *et al.*[12]

The selected temperature represents a compromise of several factors, tube heating rate, rate of atomisation of cadmium and rate of vaporisation of the sodium chloride matrix. The cadmium will atomise from the tube surface at temperatures in the 600–900 °C region,[22] but the use of 1 492 °C as the final atomisation temperature increases the rate of heating at the atomisation temperature and increases the concentration of cadmium in the tube at that time. The selection of 1 492 °C permits separation of the atomic and background signals in time at the heating rate available with the HGA-72. The faster heating rate available in the furnace used by Lundgren *et al.*[12] allowed a lower atomisation temperature to be selected in that apparatus. Optimum operating conditions will therefore be different for this analysis when performed using different designs of carbon furnace.

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# Rapid Determination of Sulphate in Natural Waters and Plant Digests by Continuous Flow Injection Turbidimetry

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The turbidimetric determination of sulphate as barium sulphate has been adapted to the continuous flow injection procedure of Růžička and co-workers. The effects of reagent composition, reagent flow-rates and length of mixing coils have been investigated. Optimum conditions were established for the determination of sulphate in natural waters and in plant digests. The results agree satisfactorily with those obtained by a standard turbidimetric method and analyses can be carried out at a rate of up to 180 samples per hour.

*Keywords*: *Sulphate determination*; *water*; *plant material*; *turbidimetry*; *flow injection analysis*

The measurement of the turbidity of a barium sulphate suspension is well known as a standard method for the determination of sulphate in natural waters,[1] and has also been used extensively for both manual and automated determinations of sulphur in soils and plants (see Beaton *et al.*[2] for a comprehensive literature review). Existing manual methods require all analytical operations to be carried out in a strictly reproducible manner, and typically not more than 10 determinations can be performed per hour.[1] In the automated turbidimetric methods, the reproducibility is secured instrumentally and the rate of analysis may typically be about 40 determinations per hour.[3]

The method of continuous flow injection analysis developed by Růžička and co-workers[4–7] has been applied successfully to spectrophotometric and/or potentiometric determinations of phosphate,[5] nitrogen[6] and chloride[7] in natural waters or pre-digested plant material at rates of up to 250 determinations per hour on a routine basis. This method utilises the rapid injection of an aqueous sample into a continuously moving carrier stream of water or chemical reagent (without air segmentation). The injected sample forms a zone that is transported towards a detector, which continuously records, *e.g.*, absorbance or electrode potential.

The purpose of this work was to show the usefulness of continuous flow injection analysis for the turbidimetric determination of sulphate and to investigate the interrelation of experimental parameters such as reagent composition, flow-rate, length of mixing coils and sample volume with the detector signal (peak height) in order to achieve optimum precision, speed of analysis and sensitivity.

## Experimental

### Reagents

All chemicals were of analytical-reagent grade.

*Poly(vinyl alcohol)*. Du Pont Elvanol 71–30.

*Hydrochloric acid*, 0.010 M.

*Barium chloride dihydrate* (5.0%) - *poly(vinyl alcohol)* (0.05%) *solution*. A 0.500-g amount of poly(vinyl alcohol) is suspended in about 100 ml of water under continuous stirring with a magnetic stirrer, then 800 ml of boiling water are added. When a clear solution is obtained, 50.0 g of barium chloride dihydrate are added. After dissolution and cooling, the solution is made up to 1 000 ml. If, in 1 or more days, a slight precipitate develops, the solution must be filtered before use.

*Standard sulphate solution*. A stock solution containing 1 000 p.p.m. of sulphate is prepared by dissolving 1.376 g of ammonium sulphate in water and diluting to 1 000 ml.

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Working solutions containing sulphate in the range 10–200 p.p.m. are prepared by suitable dilution of the stock solution.

## Apparatus and Procedure

The reagent streams are pumped by a Technicon AA II peristaltic pump using Technicon Tygon pumping tubes. The injection and mixing manifold is made from polyethylene tubing (1.0 mm i.d.) of the non-collapsible type. Mixing coils are made by winding appropriate lengths of the polyethylene tubes on glass tubes (13 or 25 mm i.d.). The injection valve and Y-shaped and straight connectors are made from Perspex blocks. All components of the manifold are conveniently fixed on a base plate by plastic blocks (Lego, Billund, Denmark).

Samples are injected manually into the carrier stream by means of disposable plastic 1-ml syringes without the hypodermic needle. If rinsed with water between injections of different samples, each syringe can be used for the injection of an infinite number of samples. Injections are made at the maximum convenient speed through a valve having a dead volume of less than 3 $\mu$l.[6] As in gas chromatography, it is important that the injections are performed reproducibly.

The turbidity is measured at 480 nm using a Beckman Model 25 spectrophotometer connected to a Beckman Model 24–25 ACC recorder and equipped with a flow-through cuvette (Hellma Type 178, light path 10 mm, volume 0.080 ml).

Depending on the analytical requirements, one of the configurations shown in Fig. 1 can be utilised. The merits of each configuration are discussed under Results and Discussion.

Water samples were injected without pre-treatment or after suitable dilution with distilled water. Plant samples were digested with nitric - perchloric acid using a Technicon BD–20 block digester and the following procedure. A 1.000-g amount of sample, previously ground and dried at 65 °C, was pre-digested with 10 ml of concentrated nitric acid at a temperature not exceeding 160 °C. Then the excess of nitric acid was driven off by adding 2 ml of con-

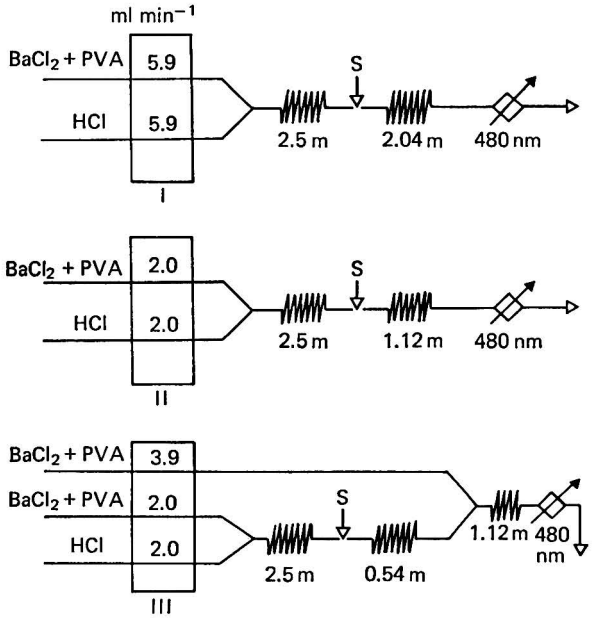


Fig. 1. Flow diagrams. Barium chloride - poly(vinyl alcohol) and hydrochloric acid are pumped jointly to form a stream into which 0.4 ml of sample (S) is injected and carried through a mixing coil towards the spectrophotometer flow cell. In configuration III more barium chloride - poly(vinyl alcohol) is added to the carrier stream after the sample has been injected.

centrated perchloric acid and increasing the temperature to 210 °C. Heating was continued until a clear, colourless solution was obtained and dense fumes of perchloric acid dihydrate appeared. After cooling, the digest was diluted with water to a specified volume of 50 ml or more.

## Results and Discussion

In the various procedures for the turbidimetric determination of sulphate as barium sulphate described in the literature, a "conditioning agent" is nearly always used in order to reproduce quantitatively the turbidities of solutions that have identical sulphate contents. These agents can be classified as follows: (1) aqueous solutions of mono- and polyvalent alcohols, such as glycerol[1]; the action of these solutions is presumably to stabilise the rate of nucleation of barium sulphate and to increase the viscosity of the solution and thereby prevent the rapid settling of barium sulphate particles; and (2) aqueous solutions of macromolecular material, such as gelatine,[3] various "gums"[8] or commercial preparations of surface-active agents[9]; the function of these compounds is probably to act as protective colloids and thereby prevent the rapid settling of barium sulphate particles.

During this study, several conditioning agents were tested. It was found that only when a protective colloid was added were reproducible turbidities obtained by flow injection analysis. Poly(vinyl alcohol) was found to be superior to gelatine, but no difference was observed between the two grades of poly(vinyl alcohol) available, namely Du Pont Elvanol 71–30 and 71–24.

In preliminary experiments, non-linear calibration graphs were frequently obtained and it was found that the shape and position of the calibration graph and the reproducibility were influenced by the reagent composition, flow-rates, volume of sample injected and the geometry of the manifold.

The results of a systematic investigation of the influence of the concentration of barium chloride and poly(vinyl alcohol) are shown in Fig. 2. It can be seen that relatively high concentrations of barium chloride produce better linearity but also give high blank readings. We believe that the high blanks are due to the difference in refractive index between reagent and reagent plus sample. A mixing boundary ("Schlieren pattern") could be seen very clearly when the flow-through cuvette was removed from the spectrophotometer and observed visually after injecting a blank or a sample. As a compromise, 5% of barium chloride dihydrate was chosen as a standard concentration. It can be seen from Fig. 2 (*a*) that the sensitivity was increased when the concentration of poly(vinyl alcohol) was increased from

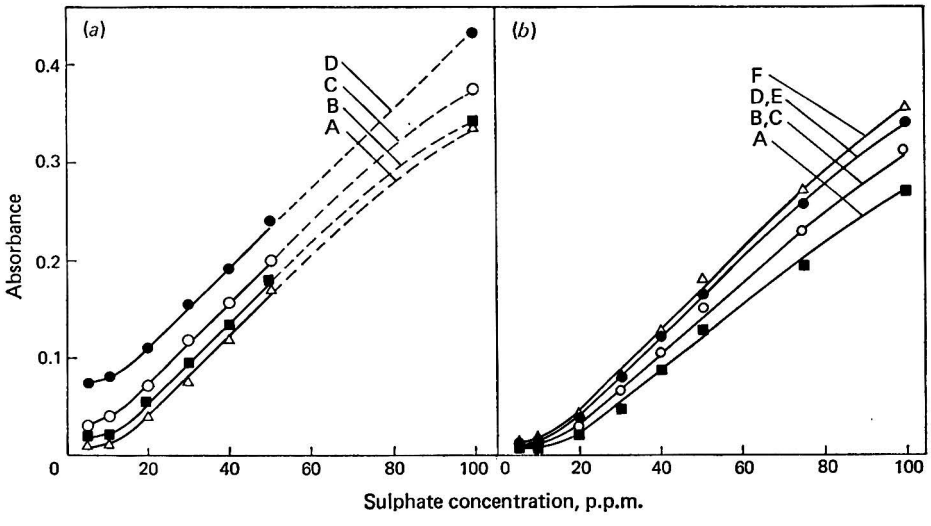


Fig. 2. Influence of reagent concentration on the calibration graphs recorded with configuration I (Fig. 1). (*a*) 0.050% poly(vinyl alcohol): A, 1.0; B, 2.5; C, 5.0; and D, 10.0% barium chloride dihydrate. (*b*) 2.5% barium chloride dihydrate: A, 0.001; B, 0.01; C, 0.02; D, 0.05; E, 0.10; F, 0.20% poly(vinyl alcohol).

0.001 to 0.2%. However, at the highest concentrations less reproducible results were obtained. Hence, 0.05% of poly(vinyl alcohol) was chosen as a standard concentration.

The calibration graphs were affected very slightly by altering the concentration of hydrochloric acid in the range from 0 to 0.1 M. When the acid concentration was increased to 0.3 M, significantly lower absorbances were recorded and the recorder base line increased considerably during the analysis of a series of standards. This effect was caused by the formation of a surface film on the windows of the flow-through cuvette, probably because the high acid concentration deactivates the protective colloid. However, perchloric acid concentrations in the standards corresponding to those present in plant digests did not affect the calibration graph when such standards were injected into a 1 + 1 mixture of 0.01 M hydrochloric acid and 5% barium chloride dihydrate - 0.05% poly(vinyl alcohol).

Using the 1-ml syringes available, it was possible to inject volumes of 0.3–0.8 ml with about the same relative precision. It was found that by increasing the injection volume from 0.4 to 0.8 ml the turbidity per unit volume injected decreased by about 25%. Therefore, an injection volume of 0.4 ml was chosen as standard.

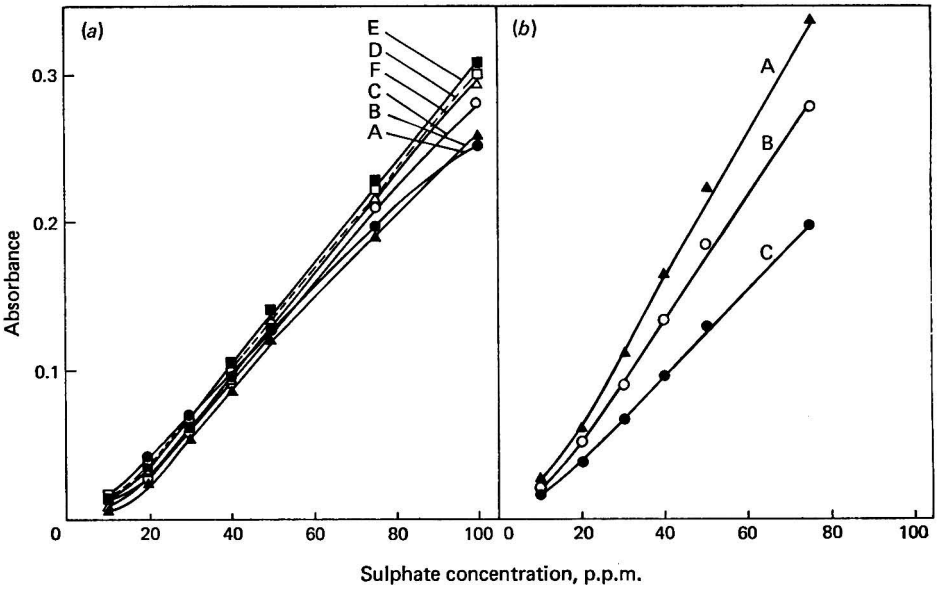


Fig. 3. Influence of reagent flow-rate and length of mixing coil on the calibration graphs recorded with configurations I and II (Fig. 1).

(*a*) 2.04-m mixing coil:

| Graph | Ba - PVA/ml min$^{-1}$ | HCl/ml min$^{-1}$ | Samples per hour |
|---|---|---|---|
| A | 2.0 | 2.0 | 85 |
| B | 2.0 | 5.9 | 120 |
| C | 3.9 | 5.9 | 140 |
| D | 5.9 | 2.0 | 130 |
| E | 5.9 | 3.9 | 150 |
| F | 5.9 | 5.9 | 180 |

(*b*) 2.0 ml min$^{-1}$ Ba - PVA, 2.0 ml min$^{-1}$ HCl:

| Graph | Length of mixing coil/m | Samples per hour |
|---|---|---|
| A | 0.70 | 130 |
| B | 1.12 | 100 |
| C | 2.04 | 85 |

Fig. 3 demonstrates the influence of reagent flow-rate and length of the reagent - sample mixing coil on the shape and position of the calibration graph. Alteration of the flow-rate of the barium chloride - poly(vinyl alcohol) reagent by a factor of nearly three produces only a slight alteration in the calibration graph and an alteration of the hydrochloric acid flow-rate has an even smaller effect. The highest speed of analysis and, surprisingly, also the best precision are obtained by using the highest flow-rates (Table I). The sensitivity and speed of analysis increase considerably when the length of the reagent - sample mixing

coil is decreased from 2 to 0.7 m. However, at the same time the precision decreases, as is the general case in flow injection spectrophotometry.[5]

It was observed (Fig. 2) that the calibration graphs were nearly flat at sulphate concentrations below about 10 p.p.m. Accordingly, 10 p.p.m. of sulphate in the injected solution is considered to be the lower limit of determination of the proposed procedure.

In Table I, continuous flow injection turbidimetry is compared with a standard turbidimetric procedure.[1] The latter procedure utilises an ethanol - glycerol - hydrochloric acid mixture as the conditioning agent and the addition of solid barium chloride dihydrate, followed by a 1-min stirring period and turbidity measurements during a further 4-min period. The maximum turbidity is recorded. The results in Table I demonstrate satisfactory agreement between the two procedures.

Using manifold configuration III, more barium chloride - poly(vinyl alcohol) reagent is added confluently to the carrier stream after the sample has been injected. With this manifold, equal (or better) precision, but lower speed and sensitivity, were obtained than with configurations I and II. As the linearity of the calibration graph was superior in the range 40–140 p.p.m. of sulphate, manifold configuration III was used for the analysis of a large number of plant digests that had sulphate concentrations within this range. A representative run is shown in Fig. 4.

TABLE I

COMPARISON OF PROCEDURES FOR DETERMINATION OF SULPHATE IN NATURAL WATERS AND IN PLANT DIGESTS

| | Standard turbidimetric method[1] (duplicate determinations) | | Flow injection (quadruplicate injections) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Manifold I | | | Manifold II | | |
| Sample | Sulphate, p.p.m. | Sulphur, % | Sulphate, p.p.m. | Sulphur, % | Standard deviation, % | Sulphate, p.p.m. | Sulphur, % | Standard deviation, % |
| *Water*— | | | | | | | | |
| PP6 .. .. | 29.0 | — | 32.5 | — | 0.13 | 32.5 | — | 1.32 |
| PP7 .. .. | 25.5 | — | 26.5 | — | 0.80 | 27.0 | — | 0.64 |
| PP10 .. .. | 46.5 | — | 45.5 | — | 0.61 | 44.0 | — | 1.06 |
| PP28 .. .. | 28.5 | — | 31.0 | — | 1.61 | 30.0 | — | 0.95 |
| *Plant digests**— | | | | | | | | |
| 9 .. .. | — | 0.73 | — | 0.71 | 0.84 | — | 0.70 | 2.04 |
| 18 .. .. | — | 0.61 | — | 0.58 | 0.24 | — | 0.59 | 0.79 |
| 39 .. .. | — | 1.08 | — | 1.05 | 0.83 | — | 1.03 | 1.77 |
| 46 .. .. | — | 0.59 | — | 0.53 | 0.41 | — | 0.55 | 1.08 |
| 50 .. .. | — | 0.73 | — | 0.73 | 1.20 | — | 0.73 | 0.71 |
| 66 .. .. | — | 0.63 | — | 0.56 | 0.92 | — | 0.56 | 1.69 |
| 21G .. .. | — | 1.07 | — | 1.07 | 1.27 | — | 1.03 | 1.54 |
| 41G .. .. | — | 0.81 | — | 0.81 | 0.70 | — | 0.77 | 1.27 |
| 47G .. .. | — | 1.09 | — | 1.09 | 1.48 | — | 1.10 | 2.92 |
| 61G .. .. | — | 0.93 | — | 1.03 | 0.26 | — | 1.03 | 2.14 |
| 157 .. .. | — | 0.48 | — | 0.43 | 1.05 | — | 0.43 | 0.59 |
| Mean (plant digests) .. | | 0.795 | | 0.781 | 0.84 | | 0.774 | 1.50 |
| Regression coefficient for calibration graph (20–70 p.p.m. sulphate) .. | | 0.9962 | | 0.9997 | | | 0.9992 | |
| Relationship between means relative to standard method .. | | 100 | | 98.2 | | | 97.4 | |

* Foliar material from cotton, *Gossypium* sp.

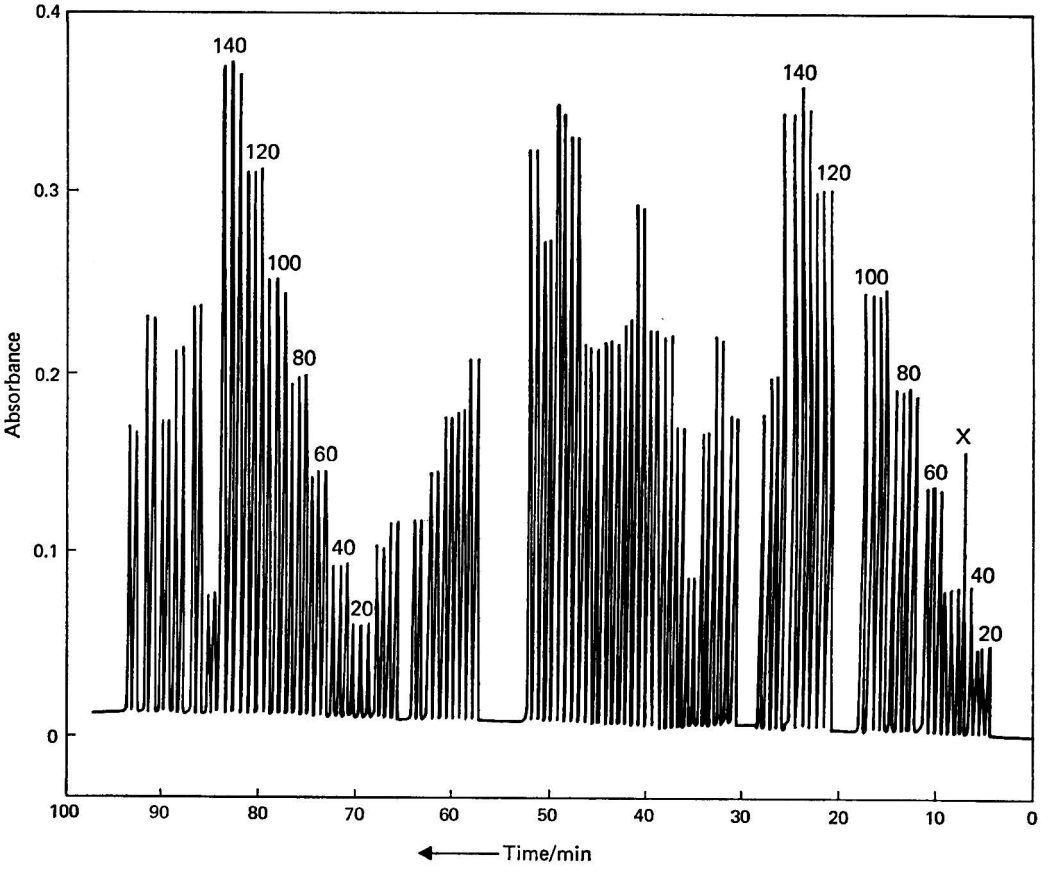


Fig. 4. Routine analyses of digested plant samples (foliar material from sugar cane, *Saccharum officinarum*) at an average sampling rate of 80 injections per hour using manifold configuration III (Fig. 1). From right to left: 20–140 p.p.m. sulphate standard solutions (corresponding to 0.033–0.233% of sulphur in plant material), each solution being injected three or four times followed by 24 samples each injected in duplicate. Then the standards are repeated and a further six samples are analysed. The peak marked X is caused by a spurious air bubble in the flow system (easily distinguished by a very abrupt increase in absorbance).

## Conclusion

The turbidimetric determination of sulphate in natural waters and plant digests can be carried out by continuous flow injection analysis at a rate of up to 180 samples per hour with a standard deviation of 0.85%. The results obtained agree satisfactorily with those obtained by was of a standard turbidimetric method.

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# Solid-state Ion-selective Electrodes for Metal Ions*

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Ion-selective electrodes for divalent metal ions ($Cu^{2+}$, $Pb^{2+}$, $Cd^{2+}$, $Hg^{2+}$) have been prepared by covering ionic conductors such as $Ag_2S$, $Ag_3SBr$ or $Ag_3SI$ with a thin metal sulphide layer. The metal sulphide layer was treated by heating under a partial pressure of sulphur. The observed standard potential is a good criterion for judging electrode performance.

*Keywords: Ion-selective electrodes; metal sulphide layers; divalent metal ions*

Pressed pellets of metal sulphide (MS) are reported to be poor ion-selective electrodes for metal ions, whereas pellets of mixed silver and metal sulphides perform well.[1–3] The standard potential of these electrodes has been calculated and shown to depend on the activity of metal (or sulphur) in the sensing material.[2,4,5]

Recently, ion-selective electrodes for halides have been developed that are based on thin silver halide layers on ionic conductors serving as supporting material.[6] In an analogous way, ion-selective electrodes for divalent metal ions can be prepared by covering ionic conductors such as $Ag_2S$, $Ag_3SBr$ or $Ag_3SI$ with a thin layer of metal sulphide.

Standard potentials that have been determined experimentally are compared with the calculated values as a check on the preparation and conditioning of the metal sulphide layer electrodes.

## Experimental

Reagent-grade chemicals and water that had been distilled in an all-silica apparatus were used. A $10^{-1}$ M solution of sodium nitrate was used for testing the ion-selective electrodes in order that they were all tested in solutions of the same ionic strength.

Supporting materials were $Ag_2S$ (Merck analytical reagent), $Ag_3SBr$ and $Ag_3SI$, prepared as described by Kennedy and Chen.[7] These materials, which were pressed into pellets with a diameter of 5 mm, were characterised by the use of common procedures such as differential thermal analysis, X-ray diffraction and electrical conductivity measurements.

Layers of CuS, PbS, CdS and HgS were deposited by various methods such as vapour deposition or wet-chemical methods, depending on the particular metal sulphide. Silver metal or carbon was used for the electrical contacts on the electrodes.

In order to obtain thermodynamically well defined metal sulphide electrodes in which there were no degrees of freedom in the sense of the phase rule, heating under a partial pressure of sulphur was applied. Sulphur was heated at 135 °C in a separate furnace, which was linked with the furnace in which the electrodes were heated (Fig. 1). The heating conditions for the electrodes (temperature and time) were different for each electrode configuration. A nitrogen flow-rate of 10 ml $min^{-1}$ was maintained through the furnaces.

The apparatus used for testing the ion-selective electrodes has been described in a previous paper.[6]

## Calculation of Standard Potentials

Different methods for the calculation of standard potentials of metal sulphide electrodes in aqueous solution have been published.[2,4,5] We have used the approach of Koebel[2] but, as our electrode configuration is different from that of other metal sulphide electrodes, a few assumptions had to be made.

For a cell of the type M - $M^{2+}_{aq.}$ - MS, the e.m.f. is given by the difference between the chemical potential of the metal in the pure metal and in the metal sulphide phase: $\mu_M(MS) - \mu_M^0 = -2FE$, where $\mu_M$ is the chemical potential of the metal, $F$ is the Faraday constant and $E$ is the e.m.f. When the chemical potential of the metal in the metal sulphide phase is known, then the e.m.f. of the cell can be calculated.

Two different electrode configurations have been prepared: carbon-contacted electrodes, C - $Ag_2S$ - MS, and silver-contacted electrodes, Ag - $Ag_2S$ - MS.

* Presented at the International Reference and Ion-selective Electrode Conference held at the University of Newcastle upon Tyne, January 7–9th, 1976.

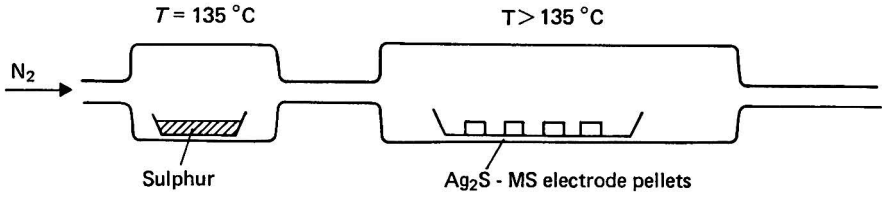



Fig. 1. Treatment of metal sulphide (MS) layer electrodes by heating under a partial pressure of sulphur.

## Carbon-contacted Electrodes

Carbon is considered to be an inert electrical contact material, which means that it will not influence the chemical potential of the components of $Ag_2S$. By heating this type of electrode under a partial pressure of sulphur the sulphur activity will become equal in the metal sulphide layer and in the $Ag_2S$ support.

For the calculations, we assume that both phases are saturated with sulphur ($\mu_S = 0$). The chemical potential of the metal in the metal sulphide phase can then be calculated from the free energy of formation of the metal sulphide; when the chemical potential of the metal in the metal sulphide phase is known, the e.m.f. of the cell can be calculated.

## Silver-contacted Electrodes

Silver metal is not an inert electrical contact material and $Ag_2S$, which is the supporting material, becomes saturated with silver when in contact with silver metal. The silver activity in $Ag_2S$ then becomes unity and the chemical potential of silver metal in $Ag_2S$ becomes zero. The chemical potential of sulphur in $Ag_2S$ is then given by the free energy of formation of $Ag_2S$. We assume that by heating the electrode under a partial pressure of sulphur the chemical potential of sulphur in the metal sulphide layer becomes equal to the chemical potential of sulphur in $Ag_2S$. The chemical potential of the metal in the metal sulphide layer can then be calculated from the free energy of formation of the metal sulphide. The calculated standard potentials of metal sulphide layer electrodes are shown in Table I.

TABLE I

CALCULATED STANDARD POTENTIAL OF METAL SULPHIDE LAYER ELECTRODES

| | Standard potential/mV *versus* N.H.E. | |
|---|---|---|
| Electrode | Carbon-contacted electrode | Silver-contacted electrode |
| $Ag_2S$ - CuS | +597 | +393 |
| $Ag_2S$ - PbS | +353 | +149 |
| $Ag_2S$ - CdS | +326 | +122 |
| $Ag_2S$ - HgS | +1090 | +885 |

## Results and Discussion

### Performance of Metal Sulphide Layers as Ion-selective Electrodes

*Ion-selective electrode for copper ions*

Copper was deposited on $Ag_2S$ by vapour deposition and was converted into copper sulphide by heating under a partial pressure of sulphur. The copper sulphide layer was shown by chemical analysis to be CuS.

The conversion of copper metal into copper sulphide is a critical process and the heating temperature and time are particularly important. Table II compares the behaviour of ion-selective electrodes prepared under different conditions. The experimental standard potential is obtained by extrapolating the e.m.f. curve to $a_{Cu^{2+}} = 1$ ($a$ = activity).

A comparison between calculated (+597 mV *versus* N.H.E.) and experimental values for the standard potential for carbon-contacted electrodes showed large deviations when the electrodes were heated at temperatures above 200 °C. At temperatures below 200 °C good

TABLE II

PREPARATION AND CHARACTERISTICS OF ION-SELECTIVE CuS LAYER ELECTRODES

| Heating conditions | | Carbon-contacted electrode | | Silver-contacted electrode | |
|---|---|---|---|---|---|
| Temperature/°C | Time/h | Standard potential/mV *versus* N.H.E. | Slope/ mV decade$^{-1}$ | Standard potential/mV *versus* N.H.E. | Slope/ mV decade$^{-1}$ |
| 180 | 7 | +582 | +28 | +482 | +27 |
| 200 | 7 | +576 | +27 | +484 | +32 |
| 220 | 7 | +466 | (+27) | +462 | +26 |
| 240 | 3 | +477 | +27 | +489 | +28 |

agreement was found: the slope of the e.m.f. curve became Nernstian and the response time was rapid (see Fig. 2).

For silver-contacted electrodes, large deviations were found between calculated (+393 mV *versus* N.H.E.) and experimental values of the standard potential. These deviations have also been found for mixed sulphide electrodes.[2]

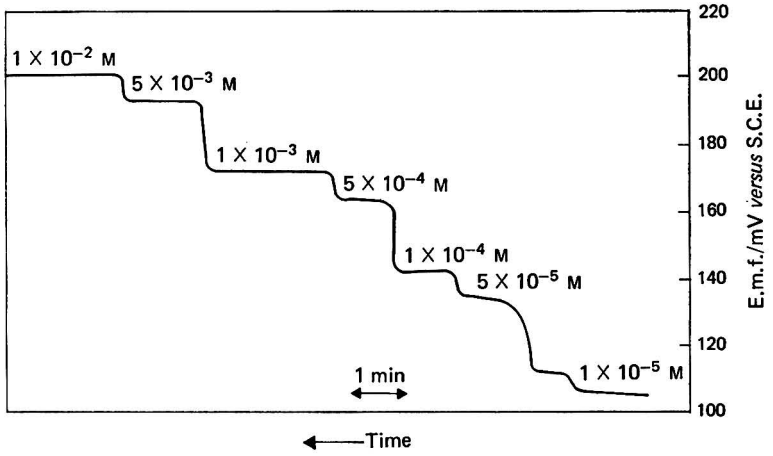



Fig. 2. Time response of a copper sulphide layer electrode to different copper(II) ion concentrations. Electrode, C - $Ag_2S$ - CuS; solution, 100 ml of $10^{-1}$ M $NaNO_3$, pH = 5, magnetically stirred.

When silver-contacted electrodes are heated at temperatures above 200 °C, other effects occur. Photomicrographs of the electrode surface (Fig. 3) show the formation of dendrites. Chemical analysis showed these dendrites to be composed of pure $Ag_2S$. Apparently, silver ions can diffuse through the CuS layer and react with sulphur vapour, leading to poor performance as an ion-selective electrode.

When $Ag_3SBr$ or $Ag_3SI$ was used as supporting material, similar results were obtained but $Ag_2S$ dendrites did not form as quickly as with the $Ag_2S$-based electrodes.

*Ion-selective electrodes for lead ions*

A PbS layer was deposited on $Ag_2S$, $Ag_3SBr$ or $Ag_3SI$ using a wet-chemical technique.[8] PbS layers deposited in this way cannot be used directly as ion-selective electrodes for lead ions. The PbS layer functions satisfactorily as a lead-selective electrode after it has been heated under a partial pressure of sulphur. Fig. 4 shows the response of a freshly precipitated PbS layer and the response of an electrode that had been heated under a partial pressure of sulphur. Electrode characteristics such as response time and slope were considerably improved by this treatment [Fig. 4 (*b*)]. Table III gives the slopes and standard potentials of electrodes heated under different conditions.

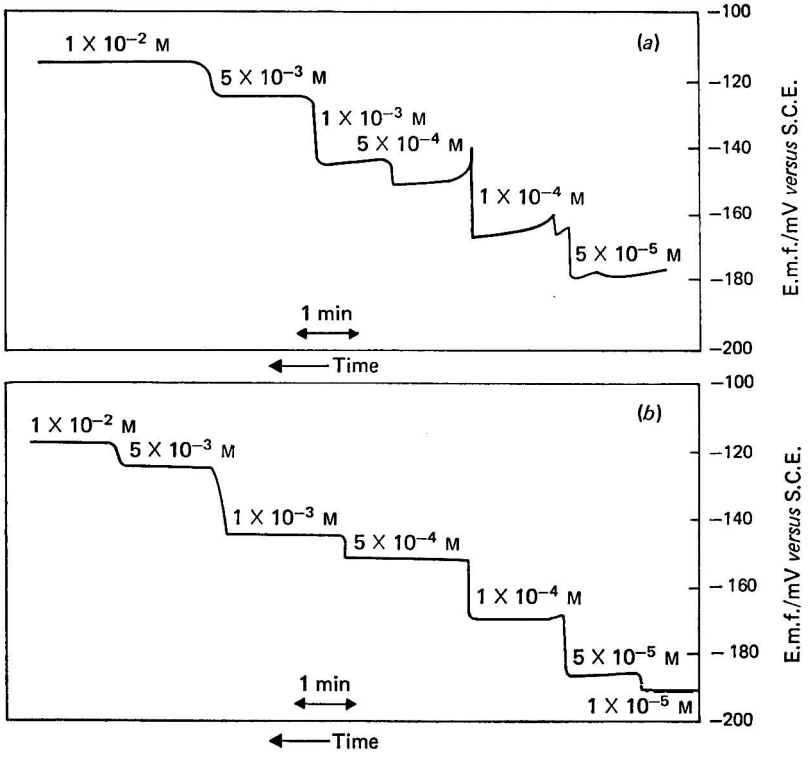



Fig. 4. Time response of a lead sulphide layer electrode to different lead(II) ion concentrations. Electrode, Ag - $Ag_2S$ - PbS: (*a*) with no treatment; (*b*) heated under partial pressure of sulphur. Solution, 100 ml of $10^{-1}$ M $NaNO_3$, pH = 5, magnetically stirred.

Different specimens of carbon-contacted electrodes showed marked differences: when the value of the standard potential corresponds with the calculated value (+356 mV *versus* N.H.E.) then the slope of the e.m.f. curve is Nernstian and the response time becomes rapid; similar observations can be made for the silver-contacted electrodes.

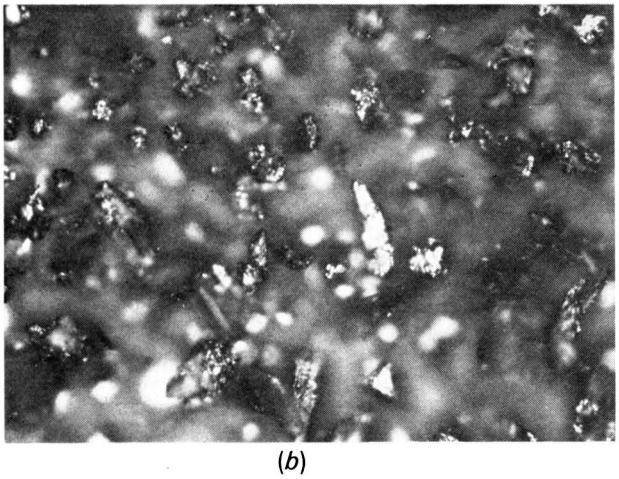
TABLE III

ELECTRODE CHARACTERISTICS OF ION-SELECTIVE PbS LAYER ELECTRODES

| Heating conditions | | Carbon-contacted electrode | | Silver-contacted electrode | |
|---|---|---|---|---|---|
| Temperature/°C | Time/h | Standard potential/mV *versus* N.H.E. | Slope/ mV decade$^{-1}$ | Standard potential/mV *versus* N.H.E. | Slope/ mV decade$^{-1}$ |
| No conditioning | | +269 | +25 | +167 | +24 |
| 180 | 3 | +350 | +28 | +137 | +20 |
| 200 | 3 | +205 | +23 | +164 | +24 |
| 220 | 1½ | +250 | +20 | +152 | +20 |
| 240 | 1 | +251 | +20 | +158 | +27 |

When the heating temperature and heating time exceed critical values, $Ag_2S$ dendrites start to grow on the surface of the PbS layer. This phenomenon is more pronounced with silver-contacted electrodes. When these dendrites are formed, the electrode shows poor performance as an ion-selective electrode for lead ions.

(*a*)



(*b*)

Fig. 3. Photomicrographs (×320) of the surface of copper sulphide layer electrodes. (*a*) CuS layer which shows good performance; (*b*) CuS layer which shows poor performance.

[*to face p. 512*

*Ion-selective electrode for cadmium ions*

By heating an aqueous solution of cadmium acetate and thiourea, a cadmium compound, probably a mixed salt or a basic salt, was deposited as a thin layer on $Ag_2S$, $Ag_3SBr$ or $Ag_3SI$. Subsequent heating under a partial pressure of sulphur resulted in the formation of a thin CdS layer.

Roughly the same conclusions as have been drawn for the copper- and lead-selective electrodes can be drawn for the CdS layer electrode.

*Ion-selective electrode for mercury(II) ions*

The HgS layer was prepared by a wet-chemical technique. $Ag_2S$ reacted with mercury(II) ions in acidic solution, resulting in the formation of a thin HgS layer on $Ag_2S$. After the electrode had been rinsed and dried it could be used as an ion-selective electrode for mercury(II) ions (Fig. 5).

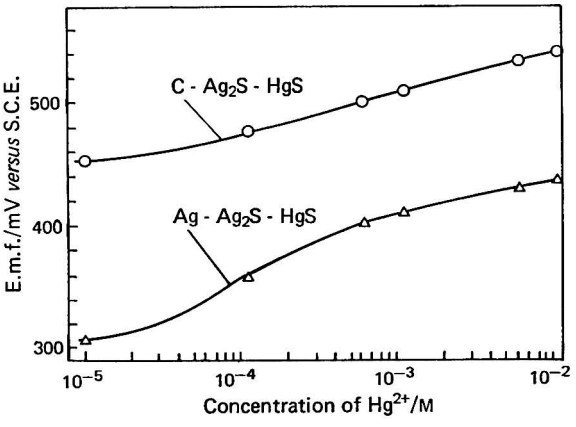



Fig. 5. E.m.f. response of $Hg^{2+}$-selective electrodes. Solution, 100 ml of $10^{-1}$ M $NaNO_3$, pH = 2, magnetically stirred.

For carbon-contacted electrodes, the observed standard potential was about +860 mV *versus* N.H.E. and the slope of the e.m.f. curve was 32 mV decade$^{-1}$. For silver-contacted electrodes, the observed standard potential was about +730 mV *versus* N.H.E. and the slope of the e.m.f. curve was 27 mV decade$^{-1}$.

The observed values for the standard potential deviated considerably from the calculated values; the reason for this discrepancy is not known. Another difference from other metal sulphide layer electrodes is that HgS layers, prepared in the above way, do not need treatment in a sulphur atmosphere.

## Criteria for the Preparation of Metal Sulphide Layer Electrodes

In the above results, there is a striking resemblance between the different thin metal sulphide layer electrodes. When the observed standard potential corresponded with the theoretically calculated value, the other electrode characteristics were good: the slope of the e.m.f. graph approached the theoretical value and the electrode response became rapid.

The chemical potential of metals in metal sulphides and hence the standard potentials of metal sulphide layer electrodes depend on the stoicheiometry of the compound. It is therefore necessary to fix the composition of the metal sulphides if reproducible electrodes are to be made. The obvious way of doing this with MS electrodes is to apply heat under a partial pressure of sulphur. The exact conditions (heating time and heating temperature) required can be found by comparing the observed and calculated standard potentials.

## Conclusion

Thin layers of metal sulphide on ionic conductors such as $Ag_2S$, $Ag_3SBr$ or $Ag_3SI$ show good performance as ion-selective electrodes for metal ions. Reproducible electrodes for $Cu^{2+}$, $Pb^{2+}$, $Cd^{2+}$ and $Hg^{2+}$ can be made in this way.

Freshly prepared metal sulphide layers, with the exception of HgS, must be treated under a partial pressure of sulphur in order to obtain the required characteristics of ion-selective electrodes.

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# Comparison of Two Different Types of Sodium Standard for Electron-probe Analysis of Soft Tissue

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Standards prepared from sodium-bearing plastic were examined with an electron probe and compared with standards prepared from freeze-dried, plastic-embedded 20% albumin solution. The calibration coefficients for sodium were $3.062 \pm 0.038$ mequiv $l^{-1}$ count$^{-1}$ $s^{-1}$ with plastic standards and $2.66 \pm 0.11$ mequiv $l^{-1}$ count$^{-1}$ $s^{-1}$ with albumin standards. The accelerating voltage was 10 kV and counting rates were normalised to $7605 \pm 29$ count $s^{-1}$ for sodium chloride crystals. The difference in calibration factors is compatible with independent measurements, which indicate shrinkage of the albumin standards as a result of freeze drying.

*Keywords*: *Sodium standard comparison*; *electron-probe analysis*; *soft tissue analysis*

Intracellular electrolyte measurements of bulk specimens are normally effected with the electron probe by comparing the characteristic X-ray intensities with prepared reference standards. Materials such as pure metals, formulated glasses, minerals and chemical salts are suitable for use as standard materials for studies of inorganic matter in that they have well understood properties, are homogeneous, are easily procured and are stable under the electron beam. Unfortunately, they differ from biological materials in mass, composition and electrolyte concentrations. The resulting large correction factors, coupled with the low relative concentrations of electrolytes in biological tissues, result in unrealistic extrapolations for the quantification of electron-probe measurements.

Standards prepared for biological studies usually include compressed pellets of organometallic compounds,[1] freeze-dried, plastic-embedded albumin samples,[2] freeze-dried membrane filters impregnated with potassium,[3] sections of methacrylate and electrolyte-bearing agar-agar,[4] collodion and alkali metal hydroxides in ethanol deposited on carbon-coated coverslips[5] and macrocyclic polyether complexes with alkali metals in epoxy resin.[6]

Fresh tissue can withstand neither the rigours of the vacuum nor the energy delivered by the electron beam; consequently, electron-probe measurements are always made on tissue samples that have been severely modified. At least one procedure, freeze drying, has been shown to result in apparent volume changes.[7,8] Hall[9] has described a procedure for the analysis of thin sections that results in some desirable simplifications for determination of the constituent elements. For the analysis of plastic-embedded samples that are infinitely thick or opaque to the electron beam, however, the selection of standards is less flexible as all volume changes must be properly accounted for in accurate analyses.

One of the early methods of preparing standards for use with thick, plastic-embedded specimens involved the use of 20% albumin samples incorporating known concentrations of the element of interest.[2] The rationale for use of this type of standard centres on the chemical similarity between albumin solutions and soft biological tissue. Distortions in the standards induced during preparation are similar to those of tissue,[7] while X-ray correction parameters for the standards are similar to those for tissue in electron-probe analysis.

With the development of macrocyclic polyether complexes incorporating alkali metals,[6] a different type of standard has become available to the biologist. These plastic castings result in higher uniformity and microscopic homogeneity than is possible with mixtures of albumin. It is the purpose of this paper to compare electron-probe measurements made on these plastic standards with data published previously for albumin standards.[10]

## Methods

A series of sodium-bearing plastic standards was prepared by the method described by Spurr[6] for potassium standards. This involved the initial preparation of a sodium - polyether complex[11] by dissolution of stoicheiometric amounts of anhydrous sodium thiocyanate (0.81 g) and dicyclohexyl-18-crown-6 (3.725 g) in 10 ml of methanol. The mixture was transferred to a vacuum oven at 70 °C for 16 h in order to remove the methanol. The complex was then introduced into a low-viscosity, epoxy resin embedding medium[12] at various levels of sodium from 10 to 1 000 mequiv $l^{-1}$. After the complex had dissolved at room temperature the mixtures were poured into embedding moulds and polymerised at 70 °C for 16 h. At levels of sodium of 400 mequiv $l^{-1}$ and below, the castings were transparent and this study was accordingly restricted to castings in the 10–400 mequiv $l^{-1}$ range. Castings with sodium concentrations above 400 mequiv $l^{-1}$ became progressively more opaque and deeper amber coloured. One surface of each casting or block was faced with a steel-knife microtome and a sodium chloride crystal and a small piece of quartz were mounted on a sample holder containing four of the standard blocks. A similar configuration was established for the second sample holder, containing another four standard samples. A thin layer of carbon was flashed over the two sample holders in a vacuum evaporator.

All electron-probe measurements were made with a 50 $\mu$m diameter, 10-kV electron beam adjusted to 50 nA on the quartz. Sodium K$\alpha$ counting rates from the crystals of sodium chloride were obtained periodically throughout the experimental sessions. As it was necessary to stabilise the plastic before collecting results, each spot was bombarded for 2 min before counting. Five random locations on each of eight standard blocks were counted for 40 s. The counting averages for each block were fitted to a straight line in order to establish a calibration graph. This graph was compared with data published previously for albumin standards.[10]

The albumin standards were prepared by adding potassium nitrate and sodium chloride to a 20% solution of bovine serum albumin. The sodium and potassium concentrations in the solution were verified with a flame photometer and the chloride concentration was measured with a Buchler - Cotlove chloridometer. Small drops of the solution were shock frozen in cooled liquid propane, freeze dried at about −80 °C, fixed with osmium(VIII) oxide vapour and embedded in a low-chlorine epoxy resin, Epon 826. The samples were faced with a steel-knife microtome, mounted in sample holders next to quartz and crystals of sodium and potassium chlorides and coated with a thin layer of carbon. Area scans of 50 $\mu$m per side were used, and care was taken not to include cracks or fissures in the embedded albumin. Data were collected using the same 2-min stabilising period for the sample and the same 10-kV, 50-nA beam parameters as were used with plastic standards. Recordings of 40-s countings were obtained from 9 to 11 locations on 11 standard blocks. Count rates for sodium K$\alpha$, potassium K$\alpha$ and chlorine K$\alpha$ radiation were monitored periodically on the pure crystals of sodium chloride and potassium chloride. All electron-probe measurements for sodium K$\alpha$ radiation were obtained with KAP and RAP diffraction crystals on an Applied Research Laboratories EMX (Model 21000) electron-probe microanalyser.

## Results

### *Plastic standards*

The calibration graph obtained from the eight blocks of sodium-bearing plastic is presented in Fig. 1. A straight-line, least-squares fit to the eight points has a slope of $13.06 \pm 0.16$ with an intercept of $288 \pm 26$ and a linear correlation coefficient of 0.999 6. From the slope, a calibration factor of $3.062 \pm 0.038$ mequiv $l^{-1}$ count$^{-1}$ $s^{-1}$ was obtained. The RAP diffraction crystal used for these measurements gave a corrected sodium K$\alpha$ counting rate from sodium chloride crystals of $7\,605 \pm 29$ count $s^{-1}$.

### *Albumin standards*

The results obtained for albumin standards are summarised in Table I. From these results a calibration factor of $9.21 \pm 0.36$ mequiv $l^{-1}$ count$^{-1}$ $s^{-1}$ was obtained. The larger relative uncertainty over those obtained from plastic standards reflects the greater lack of uniformity of the albumin samples. These results were obtained by using electron-probe operating conditions identical with those used for the plastic standards, except for the use

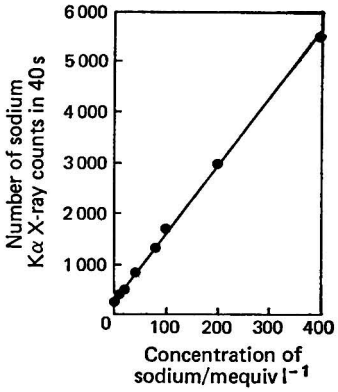


Fig. 1. Linear least-squares fit calibration graph for sodium in sodium thiocyanate - macrocyclic polyether complex in epoxy resin standards. The X-ray counts were made on blocks of the carbon-coated epoxy resin standards. $E_0$ = 10 kV, $I_s$ = 50 nA on quartz, Na Kα signal from NaCl = 7 605 count $s^{-1}$, slope = 13.06 ± 0.16, intercept = 288 ± 26, $r$ = 0.999 6.

of the less efficient KAP diffraction crystal. This decreased efficiency resulted in a corrected sodium Kα counting rate of 2199 ± 3 count $s^{-1}$ on sodium chloride. Normalisation of the results to those obtained with the RAP crystal was effected by multiplying the data by the ratio of the sodium Kα counting rate from sodium chloride crystals using the KAP diffraction crystal to the sodium Kα counting rate from sodium chloride crystals using the RAP crystal. After such normalisation, the calibration factor for the albumin standards became 2.66 ± 0.11 mequiv $l^{-1}$ $count^{-1}$ $s^{-1}$.

TABLE I

SODIUM CALIBRATION FACTOR DETERMINED WITH ALBUMIN STANDARDS

All values are listed plus or minus the standard deviation of the mean. The confidence limits on the calibration factors represent the standard deviations obtained from the square root of the sum of the squares of the standard deviations for each of the components. The corrected counting rate on a sodium chloride crystal was 2 199 ± 31 count $s^{-1}$.

| | |
|---|---|
| Sodium concentration in albumin solution/mequiv $l^{-1}$ | 127.1 ± 2.2 |
| Average radiation from 11 standard blocks/count $s^{-1}$ | 16.20 ± 0.48 |
| Off-peak background/count $s^{-1}$ | 2.40 ± 0.05 |
| Net radiation from standard blocks/count $s^{-1}$ | 13.80 ± 0.48 |
| Calibration factor/mequiv $l^{-1}$ $count^{-1}$ $s^{-1}$ | 9.21 ± 0.36 |

## Discussion and Conclusions

The calibration factor obtained with plastic standards was 1.15 ± 0.05 times larger than that obtained by using albumin standards. This difference could be explained if there had been a gross shrinkage of the dehydrated albumin standards. As these standards had been fixed with osmium(VIII) oxide, there are increased back-scattered electron losses compared with the plastic standards. However, there is insufficient osmium in the stained tissue to cause substantial changes in counting rates. If back-scattered electron losses were significant they would tend to decrease X-ray counting differences brought about by shrinkage of the albumin.

Evidence for such shrinkage has been presented earlier[7] and these results are summarised in Table II. For this earlier study a 20% albumin solution was loaded with sodium-22 and

counting rates per unit volume were obtained for embedded and fresh samples. The dehydrated, embedded albumin had a counting rate per unit volume of 1.20 ± 0.06 times that of the fresh solution. Both of these independent measurements are compatible with a 15–20% gross shrinkage of the albumin standards.

TABLE II

SPECIMEN SHRINKAGE OF FREEZE-DRIED, PLASTIC-EMBEDDED MATERIAL

| Material | Radioisotope | Number of samples | Ratio of embedded to fresh counting rate per unit volume* |
|---|---|---|---|
| Bovine serum albumin, 20% .. .. | $^{22}$Na (1.275-MeV $\gamma$) | 16 | 1.20 ± 0.06 |
| Amphiuma blood .. .. .. | $^{22}$Na (1.275-MeV $\gamma$) | 5 | 1.27 ± 0.05 |
| Mouse muscle .. .. .. .. | $^{86}$Rb (1.078-MeV $\gamma$) | 4 | 1.23 ± 0.21 |
| Mouse liver .. .. .. .. | $^{86}$Rb (1.078-MeV $\gamma$) | 3 | 1.36 ± 0.69 |

* Ratios are presented ± the estimate of the standard deviation.

The authors do not recommend one type of standard over the other. The individual investigator must determine which is most appropriate for his particular application. The albumin standards are simple to prepare and can be loaded with a large variety of elements, while the uniformity and microscopic homogeneity of plastic complexes permit the development of more precise calibration factors with plastic standards. For types of analysis that are sensitive to preparation-induced distortions, however, the accuracy of either standard is limited by our lack of understanding of the nature and extent of these distortions. The similarity of 20% albumin solutions to soft tissue samples, and the crude data that have been presented on volume changes in various types of tissue, support the concept of allowing for shrinkage of tissue by applying a correction of 15% to the calibration factor obtained with plastic standards. This would then provide a good first approximation to more accurate electron-probe measurements on thick, plastic-embedded samples.

As has been suggested previously by Ingram *et al.*,[13] it is not necessary, or even desirable, to determine new calibration factors for each tissue analysis. Pure crystals of sodium chloride can be used as secondary standards provided that the accelerating voltage is the same as was used for determining the calibration factors.

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# Identification and Determination of Alkyl Xanthate Mixtures

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A procedure for the identification and determination of alkyl xanthate mixtures based on high-performance liquid chromatography is described. The procedure is based on the oxidation of alkyl xanthates to dialkyldixanthogens. The relative retention times of the dialkyldixanthogens are given. The response of the dialkyldixanthogens is approximately proportional to the product of the concentrations of the alkyl xanthates from which they are formed. The alkyl xanthates that were studied were the ethyl, 1-propyl, 2-propyl, 2-methyl-1-propyl, 1-butyl, 2-butyl and 1-pentyl xanthates. On-column derivatisation, *i.e.*, oxidation, was found to occur under the appropriate conditions and its characteristics are discussed.

*Keywords*: *Alkyl xanthate determination*; *dialkyldixanthogens*; *alkyl xanthate oxidation*; *high-performance liquid chromatography*; *mineral processing*

Alkyl xanthates are widely used in mineral processing and the role of xanthates and related compounds as collectors in flotations has been discussed.[1,2] A number of methods, including gravimetric,[3] titrimetric,[4–11] conductimetric,[12] potentiometric,[13,14] polarographic[15] and spectrometric[16,17] procedures, have been proposed for the determination of xanthates. These methods focus on the dithiocarbonate functional group and are concerned primarily with the determination of a specific alkyl xanthate in the presence of impurities, *e.g.*, sulphides and sulphites. Methods have been suggested for the determination of dixanthogen,[18–21] which is also a significant collector in flotation. The determination of the identity and composition of mixtures of alkyl xanthates (sodium salts of alkyl xanthic acids) has largely been neglected. Rao[1] indicated that for technical-grade xanthates, it was important to identify the alkyl group. This identification has been accomplished by determination of the relative molecular mass[13] of a purified xanthate isolated from the sample. This procedure is unsatisfactory for the identification or determination of mixtures of alkyl xanthates.

Attempts in this laboratory to hydrolyse mixtures of alkyl xanthates coupled with the gas - liquid chromatographic determination of the resultant alcohols were also unsatisfactory as a means for determination of the composition of the mixtures although identification of the alkyl groups was possible. In this paper, a high-performance liquid chromatographic (HPLC) procedure is described that is suitable for the identification and determination of alkyl xanthate mixtures.

## Experimental

A Du Pont 830 high-performance liquid chromatograph with ultraviolet absorption detector was used. The column was a commercial 1 m × 0.3 cm i.d. ODS Permaphase (1% octadecylsilane bonded to a Zipax chromatographic support) packed column operated at 40 °C and 1700 p.s.i.g.

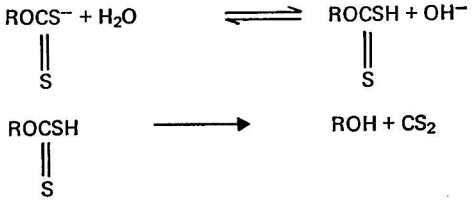
The mobile phase was a methanol - water (65 + 35) mixture. The effect of the mobile phase composition was examined.

A 20-$\mu$l volume of sample was introduced into the chromatographic system through a multi-port valve. The sodium salts of ethyl-, 1-propyl-, 2-propyl-, 2-methyl-1-propyl-, 1-butyl- and 2-butylxanthic acid were prepared. Analytical-reagent grade iodine and potassium iodide were used to prepare a potassium triiodide stock solution consisting of 0.010 M iodine in 0.10 M potassium iodide.

A stock solution of each xanthate containing 50 mg $l^{-1}$ of the sodium salt was made up and sample mixtures were prepared from these solutions. Immediately prior to the introduction of the sample into the chromatographic system, approximately 10 ml of the appropriate mixture was oxidised by the dropwise addition of the potassium triiodide solution until the triiodide colour persisted. The chromatographic injection system was flushed with the oxidised sample solution prior to the introduction of the sample on to the column.

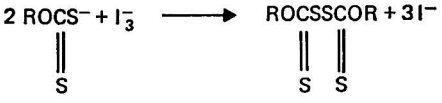
## Results and Discussion

In neutral or slightly alkaline solution, alkyl xanthates may form the corresponding xanthic acid, which will decompose to give the alcohol and carbon disulphide. Alkyl xanthates may



also be oxidised by oxygen to form the corresponding dialkyldixanthogen. The appearance

of a component in the chromatogram of a 1-propyl xanthate solution that was not present in the chromatograms of freshly prepared solutions was tentatively identified as being caused by the formation of di-1-propyldixanthogen. This suggestion was confirmed by comparison of chromatograms for the conversion of the xanthate to dixanthogen by oxidation with a potassium triiodide solution.[4] Subsequent studies on the oxidised products of solutions of

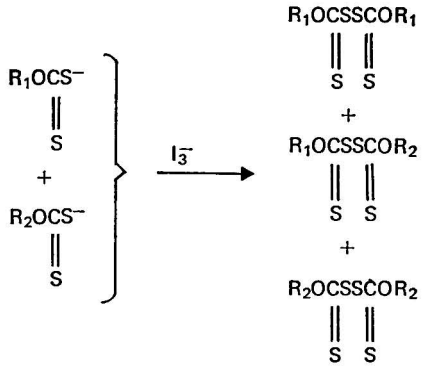


1-butyl xanthate, 2-butyl xanthate and 1-pentyl xanthate revealed that the retention times of the corresponding dialkyldixanthogens were sufficiently different to permit separation and identification of mixtures of dialkyldixanthogens.

As dialkyldixanthogens could be separated by HPLC and thus used in the identification of the alkyl xanthates in the solution prior to oxidation, the choice of mobile phase was examined in order to improve the separation. The resolution of dialkyldixanthogen improved as the proportion of methanol in the mobile phase decreased. The improved resolution was accompanied by an increase in the retention time of the components. The retention time for di-2-butyldixanthogen increased from 5.2 min for methanol - water (85 + 15) to 42 min for methanol - water (60 + 40) as the mobile phase. The major component in the mixtures used was the 2-butyl xanthate. Methanol - water (65 + 35) was chosen as the mobile phase for the separation of mixtures of dialkyldixanthogens as this mixture resolved the butyl isomers in a reasonably convenient time. Depending upon the components of the mixture, a higher proportion of methanol can be used in order to separate a larger number of samples in the same period of time.

The chromatogram of an oxidised two-component mixture of alkyl xanthates indicated that three products were formed. Two of the components had the same retention characteristics as the oxidised products of the separate alkyl xanthate solutions while the third component had an intermediate retention time. This component was assumed to be the mixed dialkyldixanthogen, $R_1OCSSSSCOR_2$, produced as shown at the top of p. 521.

Chromatograms of oxidised mixtures of 2-butyl xanthate with ethyl, 1-propyl, 2-propyl, 1-butyl, 2-methyl-1-propyl and 1-pentyl xanthates are shown in Fig. 1. The negative peak is a result of the higher proportion of water in the sample compared with the mobile phase and is an indication of the breakthrough of the unrestrained components in the sample. It is useful in determining the residence time of these components and the relative retention times of other components on the column. The retention time of the di-2-butyldixanthogen was chosen as a reference as the 2-butyl xanthate was the major component in these systems. The relative retention times for each of the dialkyldixanthogens that were examined are shown in Table I.




The relative retention time is given by

$$\frac{\theta_x - \theta_o}{\theta_r - \theta_o}$$

where $\theta_x$, $\theta_r$ and $\theta_o$ are the retention times of the component, reference and water, respectively. The reproducibilities of the relative retention times over a range of 20 samples for di-1-propyldixanthogen and 1-propyl-2-butyldixanthogen were 0.02 and 0.01, respectively. It

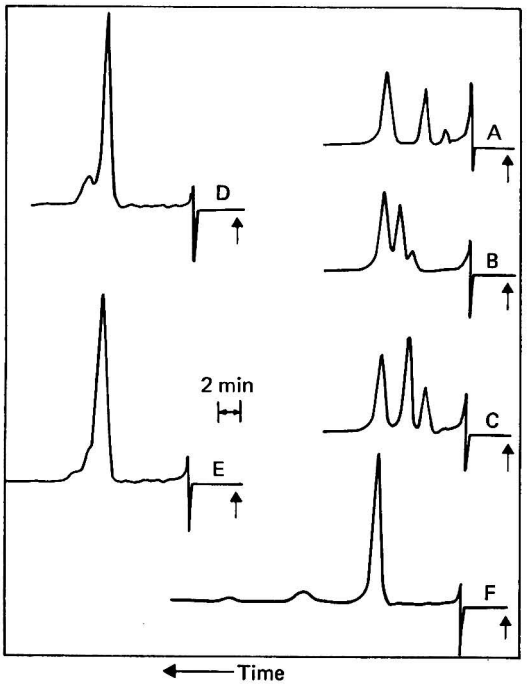



Fig. 1. HPLC separation of dialkyldixanthogens. Oxidised mixtures of 2-butyl xanthate with A, ethyl xanthate; B, 2-propyl xanthate; C, 1-propyl xanthate; D, 1-butyl xanthate; E, 2-methyl-1-propyl xanthate; and F, 1-pentyl xanthate. In chromatograms A to C, the di-2-butyldixanthogen is the last component to elute (right to left). In chromatograms D to F, the di-2-butyldixanthogen is the first component to elute after water (negative peak). The arrow indicates the time of introduction of sample.

TABLE I

RELATIVE RETENTION TIMES OF DIALKYLDIXANTHOGENS

| Alkyl components of dialkyldixanthogen | | Relative retention times |
|---|---|---|
| $R_i$ | $R_j$ | |
| Ethyl | Ethyl | 0.30 |
| Ethyl | 2-Butyl | 0.51 |
| 1-Propyl | 1-Propyl | 0.68 ± 0.02 |
| 1-Propyl | 2-Propyl | 0.57 |
| 1-Propyl | 2-Butyl | 0.82 ± 0.01 |
| 2-Propyl | 2-Propyl | 0.49 |
| 2-Propyl | 2-Butyl | 0.69 |
| 1-Butyl | 1-Butyl | 1.49 |
| 1-Butyl | 2-Butyl | 1.21 |
| 2-Butyl | 2-Butyl | 1.00 |
| 2-Butyl | 2-Methyl-1-propyl | 1.15 |
| 2-Butyl | 1-Pentyl | 1.84 |
| 2-Methyl-1-propyl | 2-Methyl-1-propyl | 1.32 |
| 1-Pentyl | 1-Pentyl | 2.75 |

was assumed, although not confirmed, that similar reproducibilities for the other dialkyldixanthogens would be obtained.

For the situation when two dialkyldixanthogens have very nearly the same relative retention times, *e.g.*, di-1-propyl- and 2-butyl-2-propyldixanthogen, identification is assisted by the presence or absence of the associated components. For example, the 2-butyl-2-propyldixanthogen cannot be present unless both the di-2-butyl- and the di-2-propyldixanthogens are also present. Similarly, if both the di-1-propyl- and di-2-butyldixanthogens are present, the 1-propyl-2-butyldixanthogen must also be present.

The quantitative response of the di-2-butyl-, 2-butyl-1-propyl- and di-1-propyldixanthogen components from oxidised mixtures of 2-butyl and 1-propyl xanthates was determined. Mixtures were prepared from fresh stock solutions to contain a total of 50 mg $l^{-1}$ of the sodium salts of the two reagents. The mass fractions of the 2-butyl xanthate in these mixtures were 0.091, 0.167, 0.286, 0.500 and 0.833. The mixtures were oxidised with potassium triiodide solution immediately prior to chromatographic analysis. Chromatograms of the resulting dialkyldixanthogens are shown in Fig. 2. The observed response of the three dialkyldixanthogens was almost proportional to the product of the appropriate mass fractions of the alkyl xanthate components of the dialkyldixanthogen in the mixture before oxidation:

$$I_{ij} \propto C_{ij} \propto (\Sigma C)\,(f_i)\,(f_j)$$

where $I_{ij}$ is the peak height of the component $R_iOC(=S)SSC(=S)OR_j$, in which $R_i$ and $R_j$ represent alkyl groups of the combining alkyl xanthates, $C_{ij}$ is the concentration of that component, $\Sigma C$ is the total xanthate concentration (which is constant in the samples used) and $f_i$ and $f_j$ are the mass fractions of the individual xanthates prior to oxidation. This expression is an approximation as the exact form involves the molar fractions and a constant total molar concentration. The use of a mass rather than a molar basis was chosen for convenience as mixtures of unknown composition could not be prepared on a molar basis without prior knowledge of the composition of the alkyl xanthate.

The response of the mixed dixanthogen is greater than that of the symmetrical dixanthogen for low mass fractions and passes through a maximum at mass fractions slightly greater than 0.5 of the compound with lower relative molecular mass, as shown in Fig. 3.

It should be noted that a linear relationship exists between the ratio of response of the mixed dixanthogen to the symmetrical dixanthogen and the ratio of mass fractions of the alkyl xanthates:

$$\frac{I_{ij}}{I_{jj}} \propto \frac{f_i}{f_j} = \frac{f_i}{1 - f_i}$$

Both the ratios $I_{ij}/I_{jj}$ and $I_{ii}/I_{ij}$ are proportional to the ratio of mass fractions, $f_i/f_j$ or $f_i/(1 - f_i)$. The first of the response ratios, $I_{ij}/I_{jj}$, is the more useful at low mass fractions of component $R_i$ while $I_{ii}/I_{ij}$ is the more useful at high mass fractions of component $R_i$. The

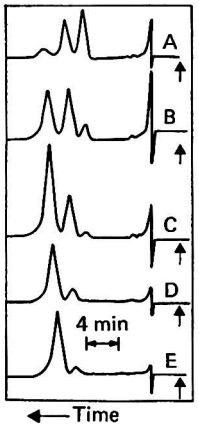



Fig. 2. Response of dialkyldixanthogens in oxidised mixtures of 1-propyl xanthate with 2-butyl xanthate. Total sodium alkyl xanthate concentration: 50 mg $l^{-1}$. Mass fraction of 2-butyl xanthate in mixture prior to oxidation: A, 0.091; B, 0.167; C, 0.286; D, 0.500; and E, 0.833.

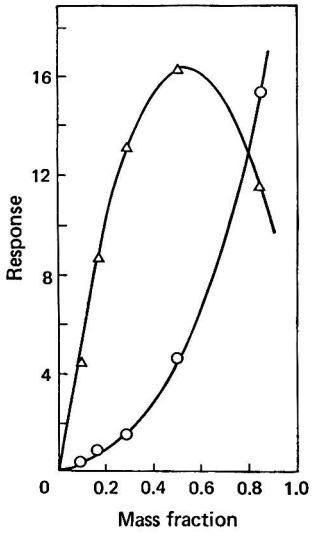



Fig. 3. Response of dialkyldixanthogens in oxidised mixtures of 1-propyl xanthate and 2-butyl xanthate. Total sodium alkyl xanthate concentration: 50 mg $l^{-1}$. △, 1-Propyl-2-butyldixanthogen; and ○, di-1-propyldixanthogen.

proportionality of the ratio of response, $I_{ij}/I_{jj}$, to the ratio of molar fractions of the alkyl xanthate can also be shown to be applicable for multi-component mixtures. The reproducibility of either ratio was approximately 5% in the 1-propyl xanthate - 2-butyl xanthate system in the mass fraction range 0.167–0.833.

No difficulty was encountered as a result of non-random combination of the alkyl xanthates in the formation of the dialkyldixanthogen. The ratio of response of the di-1-propyldixanthogen to the 1-propyl-2-butyldixanthogen was less when the sample was partly oxidised than for complete oxidation. This observation is consistent with specificity or non-random combination occurring in the oxidation of alkyl xanthate mixtures.

A cloudiness developed in some of the alkyl xanthate solutions after oxidation, showing that the solubility of the dialkyldixanthogen was exceeded. If the solutions after oxidation are allowed to stand prior to analysis, the response of the dialkyldixanthogen is less than that for solutions that are analysed immediately after oxidation. The ratio of dialkyldixanthogen to mixed dialkyldixanthogen response shows less change on standing than the individual component response, as shown in Table II.

The immediate introduction of samples into the chromatographic system gave reproducible results in spite of the potential phase instability of the oxidised system. Presumably

TABLE II

INSTABILITY OF DIALKYLDIXANTHOGEN SOLUTIONS AFTER OXIDATION

| Conditions | Response, % f.s.d.* | | | A/B |
|---|---|---|---|---|
| | Di-1-propyl-dixanthogen (A) | 1-Propyl-2-butyl-dixanthogen (B) | Di-2-butyl-dixanthogen | |
| Freshly oxidised, approximately 1 min after oxidation.. .. .. | 8.0 | 15.3 | 8.1 | 0.52 |
| 32 min after oxidation .. .. | 7.6 | 14.5 | 7.6 | 0.52 |
| 117 min after oxidation .. .. | 5.2 | 10.7 | 6.0 | 0.49 |

* Attenuation 16×.

the rapid introduction gave representative samples in spite of the inhomogeneity of the oxidised mixture. Once the sample was in the chromatographic system, the higher solubility of the dialkyldixanthogen in the methanol - water (65 + 35) mobile phase caused the second phase to re-dissolve. If a solution of lower methanol content is to be used as a mobile phase, the phase separation and its consequences would have to be examined carefully.

One consequence of the repeated introduction of samples that contained an excess of oxidant was that the column was modified. Iodine or an iodine-containing species became absorbed, probably on the stationary phase, although it is possible that adsorption occurred in the injection system. After the introduction of samples that contained an excess of oxidant, the chromatogram of unoxidised alkyl xanthate mixtures contained peaks that corresponded to the dialkyldixanthogens. These peaks were smaller than the corresponding peaks for an oxidised mixture although the ratio of responses was almost the same (0.49 compared with 0.52 for the ratio of first to second peak). Repeated introduction of unoxidised alkyl xanthate solution further decreased the peak height, indicating consumption of the oxidant present in the chromatographic system. The chromatograms of the unoxidised alkyl xanthate solutions were also characterised by the absence of the small positive peak which occurred immediately after the water peak (negative peak). These observations suggest that the process of on-column derivatisation was occurring. Because of the similarity in the chromatograms of the oxidised and unoxidised mixtures, it appears that the oxidation occurred in a small section of the chromatographic system at or near the injection point. If the oxidation occurred over the entire column, broadening of the peaks or different relative retention times would have been observed.

On-column derivatisation offers the possibility of determination of the composition of alkyl xanthate mixtures without sample pre-treatment. This modification was not developed further in this study.

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# Fluorescent Properties of Some Butyrophenones

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A spectrofluorimetric investigation of therapeutically used butyrophenones (neuroleptic drugs) is described. These substances, under certain conditions, emit fluorescent light on exposure to ultraviolet radiation.

The first part deals with the fluorescence of the butyrophenones in solution; the qualitative and quantitative aspects of the phenomenon depend on the solvent. Benperidol, droperidol, azaperone and fluanisone exhibit a distinct signal, caused by the heterocyclic substitution of the 4-aminobutyric acid chain. Qualitative tests as well as quantitative determinations were carried out, the latter requiring preliminary removal of interfering substances that absorb ultraviolet radiation. Haloperidol, bromoperidol, trifluperidol and pipamperone exhibit a weak signal.

The influence of acidity and nature of the solvent on the fluorescence was examined and the optimum pH range and the appropriate wavelengths of excitation and emission were established for the determination of each species; limits of detection are reported. Adjuvants that absorb ultraviolet radiation within the range of excitation or emission spectra need to be extracted.

The action of strong acids on the fluorescence of trifluperidol and pipamperone is discussed. Azaperone fluoresces intensely in acidic medium; direct and sensitive determinations can be performed with a limit of detection of $10^{-2}$ $\mu$g ml$^{-1}$. The drug can be made visible on chromatographic plates by its fluorescence in acidic medium.

*Keywords*: *Butyrophenones*; *drugs*; *spectrofluorimetry*

The butyrophenones[1–5] belong to a group of drugs described as neuroleptics, and show pharmacological action at low concentrations. From the analytical point of view their determination in pharmaceutical preparations can present some difficulties depending on the amount in the sample. Determinations based on the absorption of ultraviolet light, which are frequently used, are limited to minimum amounts in the microgram range.

The aim of this investigation was to determine whether the therapeutically used butyrophenones were capable of being measured by means of a fluorimetric method. As an analytical procedure the fluorescence technique is much more specific than the ultraviolet absorption method because the requirements for fluorescence emission are not only the presence of an absorbing structure but also an electronic configuration in which excited states can deactivate with emission of light. Fluorescence measurements are usually also much more sensitive because of the nature of the phenomenon and intense light sources and sensitive detectors are now available.

Butyrophenones can be considered as 4-aminobutyric acid derivatives with the general structure **I**; the most important derivatives that have been investigated are shown in Fig. 1.

The general structure of these compounds can be represented by the formula P–H, in which P is the 4′-fluorobutyrophenone moiety and H is a heterocyclic substituent replacing one hydrogen atom of the end methyl group (Fig. 1). Moiety P shows no fluorescent properties. Moiety H usually fluoresces intensely unless there is no conjugated system, as in pipamperone. Consequently, the observed fluorescence of the butyrophenones is caused by moiety H. Moiety P phosphoresces strongly (triplet state) with an emission maximum at 415 nm ($\tau = 0.004$ s).[6,7] Moiety H, when conjugated, phosphoresces with an emission maximum below 415 nm. All P–H derivatives phosphoresce at 415 nm ($\tau < 0.1$ s). The ketonic 4′-fluorobutyrophenone is described as being strongly reactive in its $\pi$-excited triplet state.[8,9]

The spectroscopic behaviour of the P–H compounds (absorption, fluorescence, phosphorescence) depends on the nature of the substituents leading to singlet - singlet and to

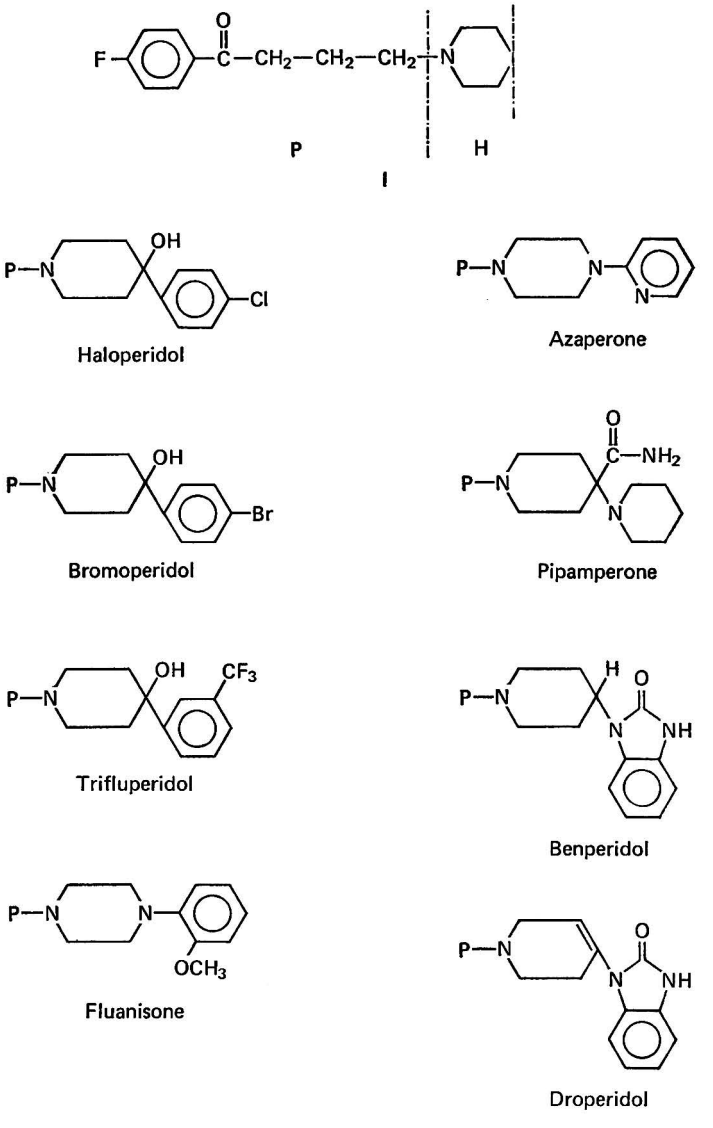


Fig. 1. General structure of butyrophenone (**I**) and structures of some important derivatives. Haloperidol, 4-[4-(4″-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone; bromoperidol, 4-[4-(4″-bromophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone; trifluperidol, 4-[4-(3″-trifluoromethylphenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone; fluanisone, 4-[4-(2″-methoxyphenyl)-1-piperazinyl]-4′-fluorobutyrophenone; azaperone, 4-[4-(2″-pyridyl-1-piperazinyl]-4′-fluorobutyrophenone; pipamperone, 4-(4-piperidino-4-carbamoyl)-4′-fluorobutyrophenone; benperidol, 4-(4-benzimidazolin-2″-one)piperidino-4′-fluorobutyrophenone; droperidol, 4-(4-benzimidazolin-2″-one)tetrahydropyridyl-4′-fluorobutyrophenone.

triplet - triplet energy transfers as well as to deactivation processes. It is probably affected by variations in the stereochemical configurations that are influenced by the substituents and by the length of the chain between P and H. A "closed" configuration with minimal distance between P and H allows triplet and singlet energy transfers; in the "open" configuration both chromophores are too far from each other to permit such energy transfers.[10,11]

## Experimental

### Apparatus

All fluorimetric measurements were carried out on an Aminco-Bowman spectrophotofluorimeter (American Instrument Co., Silver Spring, Md., USA), No. 4-8203 DE, equipped with a Hanovia 150-W xenon lamp, excitation and emission monochromators with gratings, 1 × 1 cm silica sample cells, a photomultiplier, No. R 446 S, with a high voltage of 700 V, and an *X* - *Y* recorder, the slits varying between 1 and 5 mm. No corrections were made for variations in lamp intensity or photomultiplier sensitivity.

Ultraviolet absorption spectra were recorded on a Zeiss recording spectrophotometer, Type DMR 21, equipped with a hydrogen light source, a 1-cm silica cuvette and a photomultiplier.

### Reagents

All reagents used were of analytical-reagent grade, checked before use for fluorescent contaminants and if necessary recrystallised or re-distilled.

*Acetone.*

*Boric acid solution.* 6.18 g of boric acid dissolved in 0.1 M potassium chloride and made up to 1 l.

*Chloroform.*

*Diethyl ether.*

*Dimethylformamide.*

*Ethanol.*

*Ethyl acetate.*

*Glycine solution.* 7.51 g of glycine and 5.84 g of sodium chloride dissolved in water and made up to 1 l.

*Hydrochloric acid, concentrated.*

*Propan-2-ol.*

*Methanol.*

*2-Methoxyethanol (methyl Cellosolve).*

*Potassium hydrogen phthalate.*

*Potassium chloride.*

*Potassium dihydrogen orthophosphate.*

*Propane-1,2-diol.*

*Sodium chloride.*

*Sodium hydroxide.*

*Sulphuric acid, concentrated.*

*Buffer solutions.* A series of buffer solutions having the compositions shown in Table I was prepared.

TABLE I

COMPOSITION OF BUFFER SOLUTIONS

| pH at 25 °C | KCl, 0.2 M/ml | HCl, 0.2 M/ml | KH phthalate, 0.1 M/ml | NaOH, 0.1 M/ml | $KH_2$ phosphate, 0.1 M/ml | Boric acid solution/ml | Glycine solution/ml | Distilled water/ml |
|---|---|---|---|---|---|---|---|---|
| 1.0 | 25.0 | 67.0 | — | — | — | — | — | 8.0 |
| 2.0 | 25.0 | 6.5 | — | — | — | — | — | 68.5 |
| 3.0 | — | 22.3 | 50.0 | — | — | — | — | 27.7 |
| 4.0 | — | 0.1 | 50.0 | — | — | — | — | 49.9 |
| 5.0 | — | — | 50.0 | 22.6 | — | — | — | 27.4 |
| 6.0 | — | — | — | 5.6 | 50.0 | — | — | 44.4 |
| 7.0 | — | — | — | 29.1 | 50.0 | — | — | 20.9 |
| 8.0 | — | — | — | 46.1 | 50.0 | — | — | 3.9 |
| 9.0 | — | — | — | 20.8 | — | 50.0 | — | 29.2 |
| 10.0 | — | — | — | 43.7 | — | 50.0 | — | 6.3 |
| 11.0 | — | — | — | 48.9 | — | — | 51.1 | — |
| 12.0 | — | — | — | 54.5 | — | — | 45.6 | — |

## Method

Fluorescence measurements were carried out on solutions of butyrophenones in various solvents at a concentration of 10 $\mu$g ml$^{-1}$, and in solutions prepared by mixing 2 volumes of butyrophenone solution (50 $\mu$g ml$^{-1}$) with 1 volume of buffer solution. Blank solutions were run in order to correct for any background fluorescence.

## Results and Discussion

For compounds which show weak fluorescence the maxima should be regarded as approximate. Butyrophenones are fluorescent,[12] *i.e.*, they were found to emit light on irradiation with light of wavelength below 350 nm. The intensity of fluorescence and the position of the maxima depend on the nature of the solvent used. Table II shows the excitation and emission maxima in various organic solvents in order of decreasing intensity; the butyrophenones are classified from left to right in order of decreasing fluorescence. Table III illustrates the fluorescence of the butyrophenones at different pH values.

### Table II

### Fluorescence of butyrophenones in various solvents

Concentration of butyrophenones 10 $\mu$g ml$^{-1}$; all wavelengths in nanometres.

| | Benperidol | | | Droperidol | | | Azaperone | | Fluanisone | | Haloperidol* | | Trifluperidol. HCl | | Bromoperidol* | | Pipamperone. 2HCl* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Solvent | $\lambda$ | $\lambda_{exc.}$ | $\lambda_{em.}$ | $\lambda$ | $\lambda_{exc.}$ | $\lambda_{em.}$ | $\lambda_{exc.}$ | $\lambda_{em.}$ | $\lambda_{exc.}$ | $\lambda_{em.}$ | $\lambda_{exc.}$ | $\lambda_{em.}$ | $\lambda_{exc.}$ | $\lambda_{em.}$ | $\lambda_{exc.}$ | $\lambda_{em.}$ | $\lambda_{exc.}$ | $\lambda_{em.}$ |
| Methanol | 235† | 285 | 320 | 235† | 282 | 318 | 310 | 372 | 282 | 375 | 310 | 410 | 310 | 415 | — | | 315 | 415 |
| Ethanol | 235† | 288 | 315 | 235† | 285 | 320 | 311 | 375 | 285 | 375 | 310 | 375 | 345 | 402 | 310 | 385 | 345 | 402 |
| Propan-2-ol | 235† | 286 | 320 | 235† | 285 | 325 | 310 | 375 | 290 | 373 | 310 | 390 | 309 | 400 | 310 | 385 | — | |
| Propane-1,2-diol | | 288 | 325 | | 290 | 320 | 305 | 370 | — | | — | | — | | — | | — | |
| Methyl Cellosolve | | 290 | 325 | | 290 | 320 | 315 | 380 | 290 | 370 | — | | 315 | 410 | — | | — | |
| Dimethyl-formamide | | 290 | 326 | | 293 | 325 | 315 | 390 | 295 | 370 | — | | 325 | 415 | — | | — | |
| Ethyl acetate | | 280 | 315 | | 290 | 320 | 310 | 370 | 290 | 370 | — | | — | | — | | — | |
| Acetone | | — | | | — | | — | | — | | — | | — | | — | | — | |
| Chloroform | | — | | | — | | 315 | 370 | — | | — | | 340 | 405 | — | | — | |

* Weak signal.
† Weak secondary excitation maxima.

### Table III

### Fluorescence of butyrophenones at various pH values

2 vol. of a solution of butyrophenone (50 $\mu$g ml$^{-1}$) added to 1 vol. of buffer solution. Wavelength of excitation followed by wavelength of emission.

| Solvent | Haloperidol | Bromoperidol | Trifluperidol. HCl | Fluanisone | Azaperone | Pipamperone. 2HCl | Benperidol | Droperidol |
|---|---|---|---|---|---|---|---|---|
| Methanol | Weak<br>pH 1, 2, 12<br>315; 375 nm | Weak<br>pH 1, 2, 12<br>300; 375 nm | Weak<br>pH < 7<br>350; 425 nm | Strong<br>Optimum at pH 1–2 and 6–12<br>280; 370 nm | Strong<br>Optimum at pH 1–6, max. pH 2<br>322; 398 nm at pH 2 | Very weak in all solvents including water<br>$\lambda_{exc.}$ = 330–355 nm<br>$\lambda_{em.}$ = 400–445 nm | As ethanol | As ethanol |
| Ethanol | Optimum without addition of buffer<br>310; 375 nm<br>Linear 0–50 $\mu$g ml$^{-1}$ | pH 1, 2, 12<br>295; 350 nm | Very weak<br>350; 425 nm | As methanol | As methanol | As methanol | Optimum at pH 1–2 and 6–11<br>280; 315 nm<br>No signal at pH 3–5 | Optimum at pH 7–11<br>283; 315 nm<br>No signal at pH 3–5 |
| Propan-2-ol | — | — | As ethanol | As methanol | As methanol | As methanol | As ethanol<br>283; 320 nm | As ethanol |
| Propane-1,2-diol | — | As ethanol | As ethanol | As methanol | As methanol | As methanol | As ethanol | As ethanol |
| Methyl Cellosolve | — | As ethanol | As ethanol | As methanol<br>281; 355 nm<br>Very strong | As methanol | As methanol | As ethanol | As ethanol |
| Dimethyl-formamide | — | As ethanol | As ethanol | As methanol | As methanol | As methanol | As ethanol | As ethanol |
| Acetone | — | — | — | — | Weak<br>As methanol | — | — | — |

Benperidol and droperidol fluoresce most strongly. Both molecules are substituted in the 4-position of the piperidine ring with the same heterocyclic nucleus, namely the benzimidazolin-2″-one nucleus, which is responsible for the fluorescence emission. The intensity of fluorescence is about equal for both compounds. The double bond in the tetrahydropyridyl ring system of droperidol causes a decrease in the intensity. Pipamperone possesses the weakest fluorescence capacity, which is attributed to the fact that the piperidine nucleus has no aromatic substitution.

Solutions in methanol usually provide more intense spectra than those in higher alcohols. Ethyl acetate and chloroform are less suitable solvents for fluorescence measurements. No signal can be detected in acetone solutions, even with the strong fluorescing species.

The fluorescence of azaperone is caused by the nitrogen-substituted pyridine nucleus; in fluanisone it is attributed to the substituted aminobenzene fraction. The fluorescence of haloperidol, bromoperidol and trifluperidol is less intense because of the halogen substitution.

Graphs can be prepared relating concentration and intensity of fluorescence for the strong fluorophores at the appropriate wavelengths of activation and emission.

The results of the investigation on the effects on the fluorescence characteristics of acidity and nature of solvent show that haloperidol, bromoperidol and trifluperidol emit moderate signals at different pH values. Pipamperone emits only a weak signal over the entire pH range. The remaining butyrophenones (fluanisone, azaperone, benperidol and droperidol) give a very intense fluorescence signal.

The fluorescent properties of the strong butyrophenone fluorophors have been used[12] for the determination of these compounds in pharmaceutical preparations after suitable dilution by means of buffer - solvent mixtures. The quenching interference as a result of ultraviolet absorption by the 4-hydroxybenzoic acid ester preservatives in the liquid preparations can be eliminated by extraction with diethyl ether in acidic medium. This effect can be demonstrated by examining the ultraviolet absorption spectra of the treated solutions. For each material the concentration ranges for linear response were checked before methods for their determination were worked out. The detection limit, defined as the lowest original concentration of which the fluorescence spectrum can still be recognised, is about $5 \times 10^{-2}$ $\mu g\,ml^{-1}$ for benperidol, droperidol, azaperone and fluanisone.

Trifluperidol and pipamperone can be converted into strongly fluorescing molecules by the action of sulphuric acid on their aqueous or alcoholic solutions, the spectral characteristics depending on the concentration of acid. Trifluperidol hydrochloride, for example, in water or in alcoholic solution treated with 10 volumes of sulphuric acid, yields a fluorophore with $\lambda_{exc.} = 315$ nm, $\lambda_{em.} = 435$ nm. Treatment of 5 ml of solution with 2 ml of sulphuric

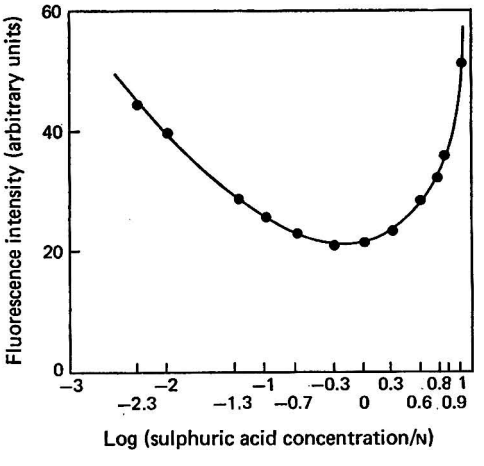


Fig. 2. Intensity of fluorescence of azaperone as a function of the concentration of sulphuric acid; $\lambda_{exc.} = 315$ nm and $\lambda_{em.} = 405$ nm.

acid produces a compound with $\lambda_{exc.} = 250$ nm, $\lambda_{em.} = 325$ nm. Pipamperone dihydrochloride in water or in an alcohol when treated with 10 volumes of sulphuric acid is converted into a fluorophore with $\lambda_{exc.} = 260$, 315 nm, $\lambda_{em.} = 420$ nm. Treatment of 5 ml with 2 ml of sulphuric acid yields $\lambda_{exc.} = 245$, 335 nm, $\lambda_{em.} = 400$ nm. In each experiment heating of the sample causes a decrease in the intensity of emission. As the intensity of emission is not constant quantitative determinations cannot be performed.

The influence of the sulphuric acid concentration on the fluorescence behaviour of azaperone is illustrated in Fig. 2. Qualitatively, the fluorescence properties remain unchanged with changes in the acid concentration; the excitation maximum is at 315 nm and the emission maximum at 405 nm.

The strong fluorescence of azaperone in acidic medium allows direct determinations to be made of azaperone preparations without removal of ultraviolet-absorbing preservatives. The limit of detection is about 0.01 $\mu$g ml$^{-1}$. Very small amounts of this substance can be detected on thin-layer plates by its intense blue fluorescence in acidic medium on irradiation with ultraviolet light.

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# SHORT PAPERS

## Optoacoustic Spectrometry of Solid Materials: Effect of the Filler Gas on the Observed Signal

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*Keywords*: *Optoacoustic spectrometry*; *filler gas*; *solid samples*; *xenon power spectrum*; *gas molecular diameter*

With the recent re-discovery of the optoacoustic effect, a simple analytical method by which to obtain the electronic transition spectra of solid and semi-solid samples has become available. A theoretical treatment based on an ideal model is currently being developed, and experimental verification is given for some of the fundamental parameters involved.[1–5] The numerous possible applications of the effect to the study of solids have generated an interest within our laboratory in setting up a single-beam, optoacoustic spectrometer system that is capable of investigating the fundamental parameters of the optoacoustic effect and of carrying out real analyses of solid samples.

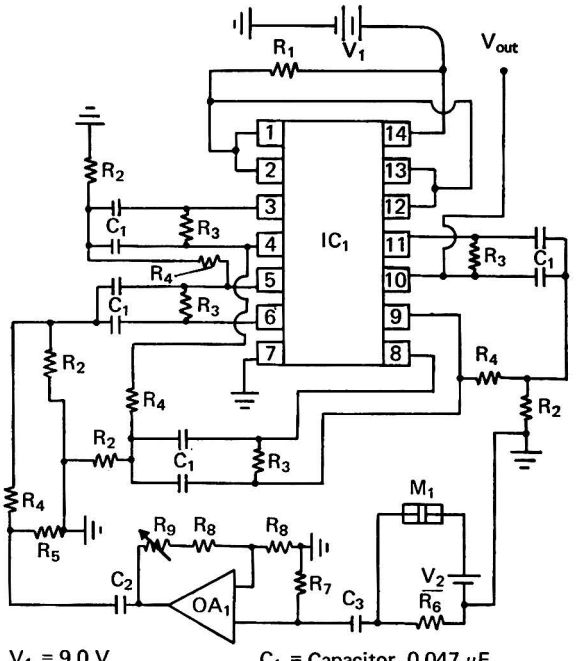
Adams *et al.*[1] have indicated that a correlation exists between the molar heat capacity of the filler gas at constant volume and the optoacoustic signal observed. Our investigation into the properties of the filler gas and its effect on the observed signal has indicated that, for the time- and temperature-independent model,[1] an additional term is needed in the equation for the relationship between the observed optoacoustic signal and the operating parameters of the sample cell system. This term should describe the efficiency of the filler gas in its ability both to take up the heat available at the surface of the solid sample and to transfer this heat as a signal between the sample and the microphone. The term involves both the ratio of the heat capacities and the molecular diameter of the filler gas. Empirical substantiation is presented by observing the change in the output signal of the microphone with a change of the filler gas; the filler gases tested are helium, hydrogen, argon, nitrogen, methane and oxygen. Helium is shown to be the most effective filler gas, providing an approximately two-fold increase in signal over that obtained with nitrogen.

### Experimental

#### Apparatus

The operational arrangement of the single-beam optoacoustic spectrometer system employed was similar to that described by Adams *et al.*[1] The cell is made entirely of optical-grade quartz and consists of a 25-mm i.d. tube, 50 mm in length, one end of which is closed by a window and the other end by a 24/40 ground-glass stopper. The detector, an electret taken from a Radio Shack, Model 33–1055, hand-held microphone, is placed within the cell as physically close to the sample as possible. The sample is attached with double-sided tape to the base of the ground-glass stopper.

The phase-sensitive lock-in amplifier fabricated was based on a design by Horlick and Betty,[6] incorporating a Signetics 565 phase-locked loop. The schematic diagram for the necessary selective pre-amplifier for the lock-in amplifier constitutes Fig. 1. This amplifier provides a pre-amplification of from $10^4$ to $10^7$ with a $Q$ of 10 for a centre band frequency of 33.3 Hz. The source was a 1 000-W, high-pressure xenon lamp from Oriel Corp., Stamford, Conn., USA. Signals were recorded on a Beckman 10-in millivolt recorder. The beam chopper was a home-made device, involving a blade with ten slots, driven by a 200 rev $min^{-1}$ constant-speed motor from W. W. Grainger Inc., West Hartford, Conn., USA. The monochromator was a 1/4-m Jarrell - Ash, Model 86–410, instrument. Filler gases of research-grade quality were obtained from Matheson Gas Products, East Rutherford, N.J., USA.




$V_1$ = 9.0 V
$V_2$ = 1.5 V
$R_1$ = Resistor, 4.06 MΩ
$R_2$ = Resistor, 5.3 kΩ
$R_3$ = Resistor, 2.03 MΩ
$R_4$ = Resistor, 101 kΩ
$R_5$ = Resistor, 470 Ω
$R_6$ = Resistor, 1.5 kΩ
$R_7$ = Resistor, 670 Ω
$R_8$ = Resistor, 1.0 kΩ
$R_9$ = Resistor, 1.0 MΩ

$C_1$ = Capacitor, 0.047 μF
$C_2$ = Capacitor, 0.47 μF
$C_3$ = Capacitor, 10.0 μF
$M_1$ = Electret microphone, 33–1055 (Radio Shack)
$IC_1$ = Quad operational amplifier, LM3900N (National Semiconductor)
$OA_1$ = Operational amplifier, No. 276–1741 (Radio Shack)

Fig. 1. Schematic diagram of the selective pre-amplifier used in observing the optoacoustic effect.

## Procedure

In order to evaluate the effect of the filler gas on the observed signal, a sample of carbon black with a surface area of 380 $m^2 g^{-1}$ (by the nitrogen B.E.T. method) was irradiated with 550-nm radiation from the xenon lamp (a point in the power spectrum at which the signal to noise ratio is high) while the filler gas was intermittently changed. For each filler gas, the analytical approach was to obtain values with the carbon black sample for the following sequence: air, sample, air, sample, air. Further, each filler gas value was corrected by subtraction of a blank with respect to any contribution of the empty (*i.e.*, containing no carbon black) cell containing the gas. To ensure complete filling of the cell with the filler gas, the observed signal was monitored until a constant value was obtained, while to ensure the complete removal of each gas, a reference air value was monitored and the cell purged until the reference value for air was obtained. A relatively constant atmospheric pressure was maintained within the cell for each filling as the cell was not pressurised by the purging procedure. The noise level was less than ±3% relative to the signal with the precision generally also being about ±3%. The absolute drift of the output signal (due possibly to a change in the flux of the xenon lamp, electronic drift, or changes in other experimental parameters) was less than ±2% relative to the total signal during the entire course of the data aquisition period, as was monitored by the reference air value.

## Results and Discussion

An evaluation of the optoacoustic system was accomplished by obtaining the power spectrum

of the xenon lamp using a carbon black sample. The quality of this spectrum (Fig. 2) led us to conclude that the apparatus was adequate for the present application.

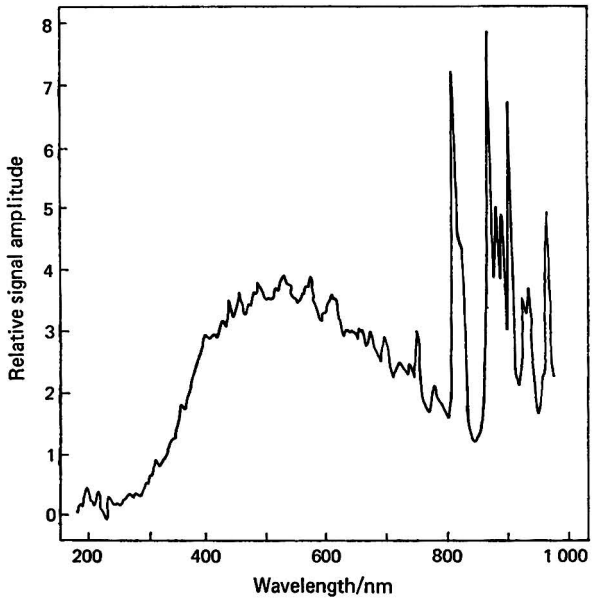



Fig. 2. Optoacoustic power spectrum of the 1 000-W xenon lamp employing a carbon black sample.

In Table I the gases used are listed, together with the following parameters: $\sigma$, the molecular diameter of the filler gas from gas viscosity data[7]; $\gamma$, the ratio of the heat capacities[8]; the theoretical $\gamma/\sigma$ value normalised such that nitrogen has a value of 1.00; and the observed $\gamma/\sigma$ value from experimental data (again normalised such that nitrogen has a value of 1.00). A graph of the normalised observed value of $\gamma/\sigma$ *versus* the calculated value has a slope of 0.97, showing a good correlation between experiment and theory.

TABLE I

CALCULATED *versus* OBSERVED VALUES OF $\gamma/\sigma$ FOR THE OPTOACOUSTIC FILLER GASES

| Gas | $\sigma$/nm | $\gamma$ | $\gamma/\sigma$ | |
|---|---|---|---|---|
| | | | Theoretical* | Observed* |
| Helium .. .. | 0.217 | 1.67 | 2.03 | 2.01 |
| Hydrogen .. .. | 0.273 | 1.41 | 1.37 | 1.51 |
| Argon .. .. | 0.366 | 1.67 | 1.21 | 1.19 |
| Oxygen .. .. | 0.362 | 1.40 | 1.03 | 1.00 |
| Nitrogen .. .. | 0.376 | 1.40 | 1.00 | 1.00 |
| Methane .. .. | 0.416 | 1.31 | 0.82 | 0.80 |
| Air .. .. | — | — | — | 1.00 |

* Normalised such that nitrogen has a value of 1.00.

An equation relating the parameters of the experimental system to the observed signal, which is independent of time and temperature, has already been proposed[1] and is represented by

$$P_{obs} = I_{o\lambda}\omega H T_\lambda s(\Omega/4\pi)(2.3\,\epsilon cl)\beta_\lambda \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where $P_{obs}$ W is the observed optoacoustic signal per pulse, $I_{o\lambda}$ W cm$^{-1}$ nm$^{-1}$ the spectral irradiance of the source, $\omega$ cm the monochromator slit width, $H$ cm the slit height, $T_\lambda$ the transmittance of the monochromator, $s$ nm the spectral band width employed, $\Omega/4\pi$ sr the

solid angle, $\epsilon$ l mol$^{-1}$ cm$^{-1}$ the molar absorptivity, $c$ the molar concentration, $l$ the sample thickness and $\beta_\lambda$ the optoacoustic efficiency factor for the sample at the wavelength specified.

Work carried out in our laboratory shows that equation (1) should contain an additional term $(\gamma/\sigma)$, which describes the ability of the filler gas to act as a signal transducer between the sample and the microphone. A theoretical basis for the term that accounts for the observed effect can be envisaged by assuming a two-stage process for signal transduction. First, in the optoacoustic signal development step, heat is generated within the sample by a radiationless energy decay mode; the transfer of this heat to the surrounding filler gas is effected by a contact mechanism. The maximum extent of contact is the minimum distance that separates the gas molecule from the surface of the sample. This degree of contact or efficiency of heat transfer is inversely proportional to the molecular diameter, $\sigma$, of the gas. In the second state of signal transduction, heat transferred to the gas from the sample surface causes a local pressure - volume change $(PV)_1$ in the gas. In comparing filler gases, the pressure - volume change $(PV)_2$ occurring at the microphone is related to $(PV)_1$ at the sample surface by $\gamma$ in an adiabatic process, where $\gamma$ is the ratio of heat capacities at constant pressure and constant volume, $C_P/C_V$, for a particular gas.

Thus, the revision of equation (1) to account for the nature of the filler gas and its empirical effect on the observed signal is represented by the last term in equation (2):

$$P_{obs} = I_{o\lambda}\omega HT_\lambda s(\Omega/4\pi)(2.3\,\epsilon cl)\beta_\lambda\,(\gamma/\sigma) \quad .. \quad .. \quad .. \quad (2)$$

The authors thank Professor David E. Wood and the Analytical Division of the Chemistry Department at the University of Connecticut for providing the necessary equipment and laboratory space needed to carry out the studies.

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# Effects of Emulsifiers and Stabilisers on the Determination of Salt in Meat Products

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*Keywords*: *Salt determination*; *meat products*; *emulsifiers*; *stabilisers*

In a paper on the determination of salt in meat and meat products,[1] it was mentioned that the method recommended by the British Standards Institution[2] occasionally failed to yield a result. The procedure concerned consists of aqueous extraction of the salt and clarification of the extract with potassium hexacyanoferrate(II) and zinc acetate, followed by Volhard titration of chloride. As is well known, the success of the last stage depends upon the coagulation of the silver chloride precipitate formed and the protection of its surface with nitrobenzene to prevent reaction with the thiocyanate titrant. In certain instances, however, it was found that the silver chloride entered into a colloidal condition and failed to coagulate, with the result that no end-point could be obtained. As there had clearly been some modification of the colloidal properties of silver chloride, it seemed feasible to suppose

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that the presence of surface-active substances might be responsible for the failure of the Volhard titration, and this view received support from the fact that all of the unsuccessful determinations involved samples that contained emulsifiers or stabilisers. Indeed, the effects of such substances on the coagulation of silver halides have been noted by several authors.[3–8] Accordingly, an investigation was mounted to determine the effects which the various types of surface-active materials commonly found in meat products might exert upon the Volhard titration. It should be mentioned that another method which has been recommended for the determination of salt[9] obviates this difficulty by oxidation of the interfering materials with potassium permanganate.

## Experimental

### Reagents

All of the inorganic reagents used were of analytical-reagent grade.

*Silver nitrate solution*, 0.1 N.

*Potassium thiocyanate solution*, 0.1 N.

*Indicator.* A saturated solution of ammonium iron(III) sulphate in water.

*Potassium hexacyanoferrate(II) solution.* Potassium hexacyanoferrate(II) trihydrate (106 g) was dissolved in water and diluted to 1 l.

*Zinc acetate solution.* Zinc acetate dihydrate (220 g) and 30 ml of acetic acid, sp. gr. 1.05, were dissolved in water and diluted to 1 l.

*Nitric acid*, 4 N.

*Nitrobenzene.* AnalaR grade.

*Sodium chloride solution, approximately* 10% *m/V.*

### Determination of Chloride in Meat

An amount (approximately 100 g) of minced beef was prepared and the chloride content determined on a 10-g aliquot in accordance with the method specified by the British Standards Institution.[2] The chloride content was found to be negligible. The determination was repeated with the addition of 4 ml of 10% sodium chloride solution immediately before the extraction stage. The whole process was again repeated with the addition not only of the salt but also of an emulsifier or stabiliser as detailed in Table I.

TABLE I

DETERMINATION OF CHLORIDE ADDED TO MEAT IN THE PRESENCE OF EMULSIFIERS OR STABILISERS

| Emulsifier or stabiliser | Supplier | Type* | Amount of emulsifier or stabiliser added, % | Chloride found as NaCl, % |
|---|---|---|---|---|
| Fixa | Rank Hovis McDougal Ltd. | PP | 0.25 | 3.7 |
| | | | — | 3.7 |
| Butterfield EP | Butterfield Laboratories Ltd. | PP | 0.25 | 3.7 |
| | | | — | 3.7 |
| MSB Sol 7 | Foodtech Ltd. | PP | 0.25 | 3.8 |
| | | | — | 3.7 |
| Admul GMS | Food Industries Ltd. | GMS | 2 | 3.8 |
| | | | — | 3.8 |
| Hymono | Food Industries Ltd. | GMS | 2 | 3.9 |
| | | | — | 3.8 |
| Sodium carboxymethyl-cellulose | British Celanese Ltd. | Carb | 2 | ~3.5 |
| | | | — | 3.8 |
| Hydroxypropylmethyl-cellulose | British Celanese Ltd. | Carb | 2 | 2.7–3.75 |
| | | | — | 3.7 |

* PP = polyphosphate; GMS = glyceryl monostearate; Carb = carbohydrate.

## Determination of Chloride in Sodium Chloride Solution

An amount of sodium chloride (4 ml of 10% *m/V* solution) was treated with an emulsifier or stabiliser as listed in Table II. The mixture was diluted to 200 ml. A 20-ml aliquot was removed by pipette and its chloride content was determined by the titrimetric procedure specified in the British Standard.[2]

Some of the surface-active substances used could be brought into solution only by adherence to the instructions provided by the manufacturers.

TABLE II

DETERMINATION OF CHLORIDE IN SOLUTION IN THE PRESENCE OF SURFACE-ACTIVE SUBSTANCES

| Emulsifier or stabiliser | Supplier | Type* | Amount of emulsifier or stabiliser added to 200 ml of solution/g | Chloride found in 200 ml (as NaCl)/g |
|---|---|---|---|---|
| Fixa | Rank Hovis McDougal Ltd. | PP | 0.025 | 0.37 |
| | | | — | 0.37 |
| Butterfield EP | Butterfield Laboratories Ltd. | PP | 0.025 | 0.37 |
| | | | — | 0.37 |
| MSB Sol 7 | Foodtech Ltd. | PP | 0.025 | 0.39 |
| | | | — | 0.39 |
| Admul GMS | Food Industries Ltd. | GMS | 0.2 | 0.38 |
| | | | — | 0.38 |
| Hymono | Food Industries Ltd. | GMS | 0.2 | 0.39 |
| | | | — | 0.39 |
| Sodium carboxymethyl-cellulose | British Celanese Ltd. | Carb | 0.2 | 0.25 |
| | | | — | 0.38 |
| Hydroxypropylmethyl-cellulose | British Celanese Ltd. | Carb | 0.2 | —† |
| | | | — | 0.37 |
| Acacia | BDH | Carb | 0.2 | No end-point |
| | | | — | 0.38 |
| Agar | | Carb | 0.2 | 0.35 |
| | | | — | 0.38 |
| Glucose | BDH | Carb | 0.3 | 0.38 |
| | | | — | 0.38 |
| Sucrose | British Sugar Corporation Ltd. | Carb | 0.4 | 0.38 |
| | | | — | 0.38 |
| Pectin | Extracted from lemons | Carb | 0.2 | No end-point |
| | | | — | 0.38 |
| Starch | Cornflour | Carb | 0.2 | —† |
| | | | — | 0.38 |
| Gelatine | BDH | Prot | 0.2 | 0.24 |
| | | | — | 0.39 |
| Albumin | BDH | Prot | 0.2 | —† |
| | | | — | 0.38 |
| Lecithin | | Ph | 0.2 | 0.39 |
| | | | — | 0.39 |

* PP = polyphosphate; GMS = glyceryl monostearate; Carb = carbohydrate; Prot = protein; Ph = phospholipid.

† End-point imprecise, value not easily repeatable.

## Discussion

As can be seen from the two tables, the surface-active materials used have been classified under five headings: polyphosphates, glyceryl monoesters, phospholipids, proteins and carbohydrates. Those members of the first three categories which were examined did not appear to cause any difficulty in the Volhard titration.

Of the proteins, only two were investigated. Gelatine occasioned the type of interference described above, while albumin reacted chemically with silver nitrate. It must be pointed

out, however, that both of these substances would be removed in the clarification stage and could not be the source of any real difficulty in the British Standard method.

Glucose and sucrose did not appear to interfere, but all of the polysaccharides tried gave rise to the unfavourable effect on the colloidal properties of the precipitated silver chloride. In addition, sodium carboxymethylcellulose and hydroxypropylmethylcellulose also complicated the clarification stage, so that great difficulties in filtration were experienced. It must accordingly be concluded that the British Standard method for the determination of chloride in meat products is inapplicable to samples containing emulsifiers or stabilisers that are polysaccharide in nature. The same applies to those meat products which contain cornflour as an ingredient.

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# Enhancement of the Sensitivity of the Cold-vapour Atomic-absorption Spectrophotometric Method Towards Mercury by Using a Sintered-glass Bubbler and a Magnetic Stirrer

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*Keywords: Mercury determination; cold-vapour atomic-absorption spectrophotometry; food analysis*

Good recoveries of several elements, with the exception of mercury, have been obtained from various organic materials after oxidation with sulphuric acid and 50% hydrogen peroxide,[1] and the Analytical Methods Committee has published two reports on the use of 50% hydrogen peroxide.[2,3] The present authors report that recoveries of mercury of more than 90% are obtained with mild digestion conditions, and the sensitivity of the cold-vapour atomic-absorption spectrophotometric technique towards mercury is enhanced by using a combination of a sintered-glass bubbler and a magnetic stirrer.

## Experimental

### Apparatus

The digestion was carried out with the apparatus described by Down and Gorsuch.[1] An

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Ekco-Electronics, Type N617, well-type thallium-activated sodium iodide scintillation counter was used at an E.H.T. of 1500 V with a 2-ml vial. An IDL 1805B five-figure scaler was used for counting. Reduction of mercury was carried out in a Quickfit MF 24/3 test-tube containing a magnetic bar with a Quickfit MF 28/3/250 domed sintered-glass bulb of porosity grade 1. A Varian Techtron, Model AA5, atomic-absorption spectrophotometer was used at a lamp current of 4 mA, slit width 300 $\mu$m and flow-rate of carrier gas (nitrogen) 1 000 ml $min^{-1}$, with an absorption cell 15 cm long and i.d. 5 mm.

## Reagents

*Mercury-203 solution.* Five millicuries of radioactive mercury were supplied as a mercury(II) chloride solution in hydrochloric acid, which was diluted with water to give count rates between 50 000 and 90 000 per 5 min in a 2-ml aliquot of the standard solution.

*Stock mercury solution,* 10 mg $l^{-1}$. Mercury(II) chloride (0.1354 g) was dissolved in water and the volume made up to 100 ml. A 1-ml volume of the solution was diluted with 20 ml of sulphuric acid (1 + 1) and the volume made up to 100 ml.

*Standard mercury solution,* 0.1 mg $l^{-1}$. To 1 ml of the stock mercury solution were added 20 ml of sulphuric acid (1 + 1) and 2 ml of 2.5% *m/V* potassium dichromate solution,[4] and the volume was made up to 100 ml.

*Calibration solutions.* Aliquots (up to 15 ml) of the standard mercury solution were diluted to 100 ml by adding 20 ml of sulphuric acid (1 + 1) and 2 ml of 2.5% *m/V* potassium dichromate solution and making up the volume to 100 ml.

*Hydrogen peroxide,* 30% *m/V*. This solution should be stored in a refrigerator.

*Tin(II) chloride solution,* 10% *m/V*.

## Procedure for Recovery of Mercury

A 1-ml volume of the mercury-203 solution was added to a round-bottomed flask containing 1 g of organic material.[1] After the apparatus had been assembled, the flask was cooled in an ice - water bath and 10 ml of sulphuric acid (sp. gr. 1.84) were added. The flask was heated with an electric heating mantle until the organic material had charred completely. The heater was removed from the flask, using heat-resistant gloves, and the flask was then immersed in the ice-water bath. About 6 ml of cold 30% *m/V* hydrogen peroxide were added dropwise and mixed thoroughly with the charred contents. The flask was replaced on the heater and heated slightly and, once the hydrogen peroxide began to decompose, the reaction proceeded without further heating. After the decomposition had ceased, the flask was heated gradually until the contents became black. The distillate in the reservoir was collected, then 2 ml of hydrogen peroxide were added and the above procedure was repeated. After the contents had become clear, the flask was cooled and the contents were transferred into a calibrated flask and diluted with water to 100 ml. Counting of the radioactivity was carried out by the procedure described by Down and Gorsuch.[1]

## Reduction and Collection of Mercury for Cold-vapour Atomic-absorption Spectrophotometry

A schematic diagram of the aspiration apparatus is shown in Fig. 1. A 2-ml aliquot of the calibration solution was added to the reduction vessel, followed by 2 ml of 10% *m/V* tin(II) chloride solution. The sintered-glass bubbler was inserted and the solution stirred for 2 min with the magnetic stirrer set at the maximum speed. The three-way stopcock was turned on to the bubbler so that the mercury vapour passed through the absorption cell and, after the peak had reached a maximum, it was turned to the by-pass. Nitrogen flowed continuously through the cell in order to prevent any condensation within the cell. The reduction vessel was emptied and the bubbler was washed with about 2 ml of 10% *m/V* nitric acid inside the vessel by stirring and bubbling nitrogen for a short period.

# Results and Discussion

## Recovery of Mercury

In duplicate tests with 1.0-g samples of sucrose, cocoa and butter, the recoveries of mercury

were as follows: sucrose, 89.3 and 90.7%; cocoa, 91.2 and 96.5%; and butter, 91.1 and 96.2%. Hence for these types of samples recoveries of more than 90% can be obtained.

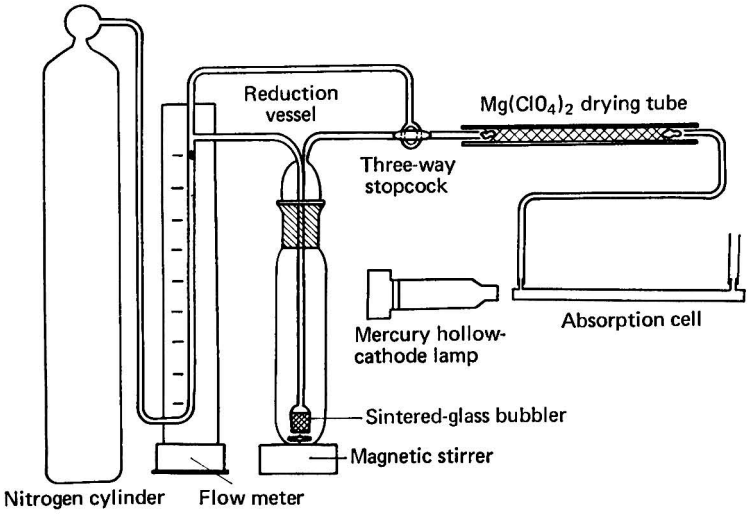


Fig. 1. Aspiration apparatus for the cold-vapour technique.

## Decomposition of Samples

The decomposition of hydrogen peroxide is an exothermic reaction[5] and, if concentrated hydrogen peroxide is added to the hot charred material, it decomposes violently. In the present procedure, cold 30% *m/V* hydrogen peroxide is added to the cold, charred sample in an ice - water bath and, under these conditions, only slight decomposition of the hydrogen peroxide occurs. Upon heating the mixture slightly, decomposition of hydrogen peroxide begins and the reaction then continues without further heating, so that the digestion proceeds smoothly and effectively without any hazards. The amount of hydrogen peroxide used and the loss of mercury can be kept to the minimum.

## Enhancement of the Sensitivity Towards Mercury

The sensitivity of cold-vapour atomic-absorption spectrophotometry is proportional to the number of atoms that enter the absorption cell. In this work, the contents of the reduction vessel were stirred magnetically before aspiration, and most of the reduced metallic mercury

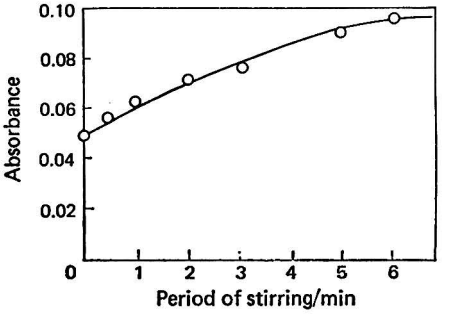


Fig. 2. Effect of stirring time on the peak height. The standard solution containing 30 ng of mercury was reduced with 2 ml of the 10% tin(II) chloride solution.

was found to be collected in the pores of the sintered-glass bulb. It was ascertained that the peak height was reduced by approximately 20% when the contents of the vessel were discarded before aspiration. The mass corresponding to 1% absorption was found to be 3 ng of mercury, compared with about 10 ng when a capillary nozzle bubbler was used instead of the sintered-glass bubbler and magnetic stirrer.

The calibration graph was linear up to 30 ng of mercury. Higher peaks were recorded as the period of stirring increased (Fig. 2). After 6 min the response was about twice the original height and reached a plateau. However, in the light of experience, the period of stirring was set at 2 min for the present work. On increasing the flow-rate of carrier gas, the peaks became progressively sharper and higher.

There was no interference from undecomposed hydrogen peroxide up to a concentration of 1.0% *m/V*.

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# Spectrophotometric Determination of Malonate with 4-Hydroxybenzaldehyde

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*Keywords*: *Malonate determination*; *4-hydroxybenzaldehyde*; *spectrophotometry*

Although malonate has been proved to be a common compound in living organisms, participating in many pathways of metabolism, there are few methods for its identification and determination.[1]

While investigating the colour reaction of many organic compounds, especially acids, with 4-hydroxybenzaldehyde, Mossor[2] found that a group of aromatic and cyclic acids (4-hydroxy and 4-methoxy derivatives of cinnamic, shikimic, usnic and uronic acids) gave a deeply coloured reaction product and that some aliphatic dicarboxylic acids with three or four carbon atoms, namely malonic, oxaloacetic, acetoacetic and tartronic acids, produced a purple dye. The reaction between 4-hydroxybenzaldehyde and shikimic acid has been used for the determination of this acid.[3] These observations suggested a simple method for the determination of malonate; the main difficulty lay in the removal of the acids that produced the deeply coloured material that screened the weaker colour of the malonate, and separation of malonate from other aliphatic acids that gave a positive reaction under the same conditions.

## Experimental and Results

### Reagents

Malonic acid was provided by Sojuzchimexport, USSR; oxaloacetic and shikimic acids were obtained from Sigma Chemical Company, USA; tartronic (hydroxymalonic), glucuronic and galacturonic acids from Calbiochem, USA; *p*-coumaric, caffeic and ferulic acids from

Koch-Light Laboratories, England; sinapic acids from Th. Schuchardt, West Germany; and chlorogenic acid from C. Roth, West Germany. Other chemicals were Polish reagents of the purest commercial grade obtainable. Acetoacetic acid was prepared by alkaline hydrolysis of ethyl acetoacetate.[1] Unless otherwise stated, the reagents were used without pre-treatment.

## Details of the Reaction

The starting point of the investigation was the method for the determination of shikimic acid.[3] The conditions that could influence the intensity of the colour produced by malonate were examined, namely concentration of sulphuric acid, concentration of 4-hydroxybenzaldehyde solution, temperature and time of heating of the sample in the water-bath, period from the end of heating until measurement and stability of the colour with time.

The optimum conditions proved to be the same as for shikimic acid,[3] *i.e.*, concentration of sulphuric acid, about 73%; concentration of 4-hydroxybenzaldehyde solution, 2 mg ml$^{-1}$; time of heating in a boiling water bath, 60 min; the colour reached maximum intensity after 10 min and remained stable for at least 2 h. There was a slight decrease in the absorbance of the more concentrated solutions of malonate after 24 h, but at lower concentrations no changes were observed.

Thus the procedure recommended for development of the dye with malonate is almost the same as that for shikimic acid. The absorption graph of the coloured product of malonate and 4-hydroxybenzaldehyde has one maximum, at about 560 nm (Fig. 1).

As a positive reaction with 4-hydroxybenzaldehyde is given by numerous compounds, *e.g.*, aldehydes, lower alcohols, sugars, uronic acids, 4-hydroxy and 4-methoxy derivatives of cinnamic acid, shikimic acid and some acyclic dicarboxylic acids (oxaloacetic, acetoacetic and tartronic),[2,3] the sample must be purified to remove the interfering compounds. Several of these compounds are removed in the course of the standard procedure for extraction and purification of the sample. Cinnamic acids are held on a cation-exchange resin.[4–7] The non-acidic compounds (sugars and lower alcohols) are removed by passing the solution through an anion-exchange resin, on which only the acidic compounds are retained.

Shikimic and uronic acids are eluted from the anion-exchange column with 3–3.5 N acetic acid,[3,8] while malonate remains on the column and can be eluted with a significantly higher concentration of the acid.[8] As the purest commercial acetic acid contains trace amounts of aldehydes, and produces a colour under the described conditions, it has to be purified before use by boiling under a reflux condenser with chromium(III) oxide for 3 h. The acid is then distilled and the first 80% of the distillate collected. Tests were not carried out to see if oxaloacetic, acetoacetic and tartronic acids were eluted from the anion-exchange column

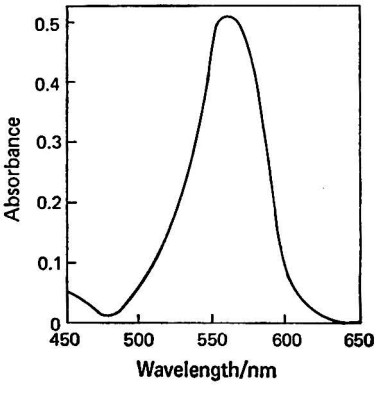


Fig. 1. Absorption graph of dye produced in reaction of malonate (0.6 μmol ml$^{-1}$) with 4-hydroxybenzaldehyde.

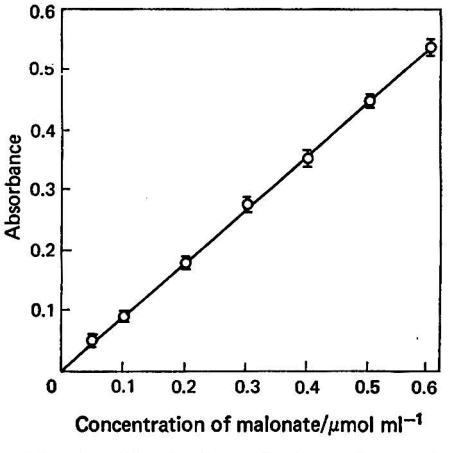


Fig. 2. Standard graph for malonate in reaction with 4-hydroxybenzaldehyde. The points are the average of five separate measurements, the scattering being indicated.

as they are completely removed by heating the sample for 5 min at 100 °C. All of these procedures have no effect on the malonate.

### Recommended Procedure

The test solution is purified by passing it through a cation-exchange resin, then transferred to an anion-exchange resin column (10 cm × 1 cm i.d. containing Dowex 1-X10 resin). Shikimic and uronic acids are eluted with 50 ml of 3.5 N acetic acid and the malonate is then eluted with 100 ml of 5.5 N acetic acid. This effluent is evaporated to dryness at 60 °C, the residue dissolved in 3.0 ml of distilled water and the solution heated for 5 min in a boiling water bath, then cooled. A 1-ml aliquot of the solution is taken and 3.0 ml of concentrated (96%) sulphuric acid are added with continuous stirring. After cooling the solution, 1.0 ml of a 0.2% aqueous solution of 4-hydroxybenzaldehyde is added and the mixture heated for 60 min in a boiling water bath, then cooled and made up to 5.0 ml and allowed to stand for 10–15 min. The absorbance of this solution is measured at 560 nm. A standard graph for malonate is shown in Fig. 2. This procedure is suitable for the determination of malonate in samples down to 0.05 $\mu$mol ml$^{-1}$. The method is used in our laboratory for the determination of malonate in many plant extracts and no difficulties have been experienced even with complex extracts.

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# Double Peaks in the Atomic-absorption Determination of Lead Using Electrothermal Atomisation

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Lead atomisation peaks in atomic-absorption spectrophotometry with electrothermal atomisation are subject to enhancement or attenuation, and also to temporal displacement, if the sample matrix contains interfering metals or organic residues. Regan and Warren[1] have reported the inter-element effects of mixing alkaline earths with aqueous lead nitrate or chloride solutions. With the sole exception of magnesium in lead nitrate solutions, their results, acquired by use of Perkin-Elmer HGA-72-403 apparatus, showed a diminution in the peak heights for absorption by lead. However, if organic materials, particularly ascorbic acid, were added to the samples, the lead peaks were heightened above the values obtained for pure lead nitrate - chloride solutions. Also noticeable in their results was an accompanying shift to earlier appearance times for the lead peaks with organic additives, although they made no comment on this feature. During the examination, in this laboratory,[2] of some

freshwater mussel residues in order to determine trace elements, the lead peaks showed some interesting similarities to those obtained by Regan and Warren. Further work on aqueous solutions of lead, reported here, produced evidence of twins in the atomisation peaks.

## Experimental

The instrument[2] used was a single-beam spectrophotometer assembled from the following components: hollow-cathode lamp and stabilised supply; PAR, Model 125, sector disc; Perkin-Elmer, Model HGA-2000, Massmann-type graphite atomiser and electrical supply unit; Jarrell - Ash 0.5-m Ebert monochromator, with curved bilateral slits and a grating blazed for maximum energy at 250 nm; 1P28 RCA photomultiplier; PAR, Model 122, lock-in amplifier with a time constant of 0.01–0.03 s; Biomation, Model 1015, four-channel transient recorder, and memory unit, using 1024 10-bit words per signal. Quartz optics were used for focusing.

This system was tested for absorption peak distortion and truncation with the most rapidly volatilised elements, cadmium and lead. Owing to the very small time constant used, with the consequent wide band width, the signal trace showed no significant distortions, unlike the situation occurring with direct input to a conventional pen recorder.[2,3] For background absorption measurements, the hollow-cathode lamp was replaced with a deuterium arc continuum source; however, the background effects from aqueous lead nitrate solutions proved to be negligible. Solutions containing relatively large amounts of added ascorbic acid produced a small background absorption. This absorption was successfully eliminated by increasing the charring cycle to 40 s. Solutions were pipetted by use of an Eppendorf pipette through the central port of the graphite furnace in 25-$\mu$l aliquots. The samples were dried at 150 °C for 30 s, charred at 500 °C for 30–40 s and then atomised at 2 200 °C for 15 s. The tube was purged with nitrogen at a flow-rate of 24 ml $s^{-1}$ continuously.

## Results and Discussion

The traces in Fig. 1 [(*a*)–(*i*)] are the lead atomisation peaks at a wavelength of 283.3 nm obtained from various lead solutions. Lead from lead nitrate solutions acidified with nitric acid normally develops a single peak [Fig. 1 (*a*)], but with the addition of 1% *m*/*V* ascorbic acid a doubling occurs and a new, earlier atomised maximum becomes evident [Fig. 1 (*b*)]. Twinning also occurs when hydrofluoric acid is added to lead nitrate solutions [Fig. 1 (*c*)] or when it is used as part of the digestion procedure for organic solids [Fig. 1 (*h*) and (*i*)]. In both instances the early peak is developed reproducibly, but is not emphasised.

All those solutions containing hydrofluoric acid retained a greater proportion of lead in the later atomisation peak with an appearance time nearly coincident with that of the single lead nitrate peak. The doubling of the trace was also evident when the breakdown of organic solids in nitric acid and hydrogen peroxide solution (to carbon dioxide and water) was incomplete and a considerable soluble organic matrix resulted. Samples of NBS standard material No. 1571 (orchard leaves) were digested with nitric acid and hydrogen peroxide solution at 130 °C in PTFE bombs and diluted with doubly distilled water. The two lead peaks obtained with this treatment were almost identical [Fig. 1 (*d*) and (*e*)], the later (5.7 s) peak maintaining a slight supremacy in height. However, if ascorbic acid was added to these solutions, the early peak was enhanced at the expense of the later peak [Fig. 1 (*f*)].

A barely discernable shoulder on the leading edge is all that is observable on the vaporisation of an aqueous solution of seston (aquatic samples containing suspended particulate matter) [Fig. 1 (*g*)]. These solids were isolated by centrifugation from large volumes of the natural waters taken from the Kingston basin of Lake Ontario; they were partially dissolved by treatment with nitric acid and hydrogen peroxide. Evidence of an early atomisation peak in traces given by these samples might easily be overlooked or dismissed. Most of the lead absorption appears at a time (5.7 s) almost identical with that observed with pure lead nitrate solutions. When hydrofluoric acid is used in the digestion procedure in order to effect complete dissolution, however, a pronounced first peak is produced [Fig. 1 (*h*) and (*i*)].

The question of whether either peak in the lead absorption trace from any of these synthetic or natural samples was caused by molecular absorption or scattering was fully investigated. This was found not to be the case. No background was observed using the deuterium continuum source at either appearance time. Measurements were made at

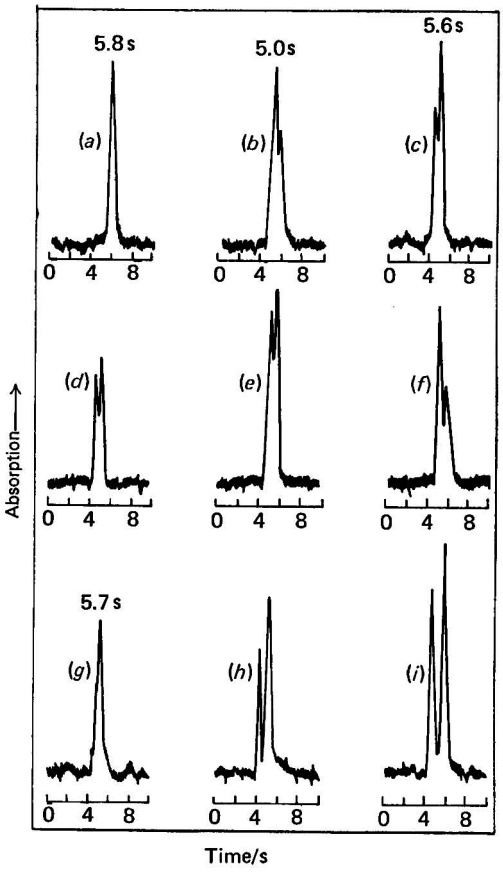


Fig. 1. Atomisation peaks at a wavelength of 283.3 nm and a temperature of 2 200 °C from various aqueous solutions: (*a*) lead (1.75 ng) in solution in 5 × $10^{-2}$ M nitric acid; (*b*) lead (1.75 ng) in 1% ascorbic acid and 5 × $10^{-2}$ M nitric acid solution; (*c*) lead (1.75 ng) in 5 × $10^{-2}$ M nitric acid with 2 drops (about 0.05 ml) of 42% hydrofluoric acid added in a volume of 50 ml; (*d*) lead (1.65 ng estimated) in 0.073 2 g of orchard leaves digested with 2 ml of nitric acid and 1 ml of 30% hydrogen peroxide solution, diluted to 50 ml with water; (*e*) lead (4.33 ng estimated) in 0.192 0 g of orchard leaves in 50 ml of solution, with the same digestion as in (*d*); (*f*) lead from the same sample as (*d*) but with 1% ascorbic acid added; (*g*) lead in seston sample (0.081 2 g), with the same digestion as in (*d*); (*h*) lead in the same seston sample as (*g*) but with 0.2 ml of hydrofluoric acid added during digestion; and (*i*) lead in seston sample (0.099 4 g), with the same digestion as in (*h*).

increasing slit widths up to 200 $\mu$m and centred at a wavelength of 283.3 nm. Some additional experiments to search for absorption at nearby wavelengths (within 5 nm) were conducted. In both situations no absorption was found. There is no doubt that each trace resulted from an atomic absorption and exclusively that of lead.

The interferences caused by the additives, ascorbic acid, hydrofluoric acid and perhaps powdered graphite (see below) are generally beneficial. In most instances they provide a

considerable enhancement of the absorption signal. The second feature, earlier vaporisation (perhaps sublimation or dissociation) of the element, implies that different charring mechanisms or residues result when these additives are used. Some experiments were conducted with the furnace cycled only through the drying and charring stages and charged with serial 100-$\mu$l aliquots of lead nitrate. Up to 1.2 mg of lead nitrate were delivered and the furnace tube was broken in order to recover the residue. These experiments proved unsuccessful owing to the thermal spreading of the charge and the porosity developed by the graphite walls. Subsequent X-ray powder diffraction analysis of the residue showed only a graphite pattern.

An attempt to simulate the charring cycle of the HGA-2000 was conducted in a muffle furnace operating at 450 °C in an atmosphere of nitrogen. Large amounts (about 10 g) of pure lead nitrate and lead nitrate containing 10% *m/m* of ascorbic acid were heated for 1 h in graphite crucibles. This time was considerably longer than the 20–40-s heating time in the Massmann furnace and, moreover, the ascorbic acid to lead ratio was only about 1 : 10 (compared with about $10^5$ : 1 in the Massmann furnace). Powder patterns of the residues confirmed the presence of both dimorphic forms of lead(II) oxide, red litharge and yellow massicot. When pure lead nitrate was reduced, a thin layer of the yellow oxide was formed adjacent to the crucible walls, while the central bulk formed red litharge exclusively. However, the sample with added ascorbic acid produced massicot in the central core (up to 40%) as well as along the crucible walls; the influence of the organic matrix was similar to that of the carbon in intimate contact with the outer surfaces.

It was obviously difficult to reproduce accurately the surface - sample interface of the HGA-2000 in the muffle furnace, so that these experiments were designed to produce the most stable form of oxide (litharge). That large amounts of thermally unstable massicot were actually formed is indicative that similar results may obtain in a Massmann furnace, perhaps to a lesser degree.

The inference drawn here is that both dimorphs of lead(II) oxide are formed during the charring step in the electrothermally atomising atomic-absorption spectrophotometer. Moreover, the presence in the sample matrix of variable amounts of organic materials or modifiers such as hydrofluoric acid and powdered carbon influences the ratio of massicot to litharge. Other metals in the matrix that compete for oxygen in the reducing stages are also likely to control the ratio. The orchard leaves contain 2% *m/m* (dry mass) of calcium and the presence of such an amount of this element is known[4] to interfere in the determination of lead, producing displaced and shouldered peaks. The absolute coincidence of the earlier atomised peak with the reduction of lead residues to the yellow oxide is not proved here, but there is an indication that this relationship exists.

The reaction mechanism of the charring process can only be a subject for conjecture at the moment but there is no doubt that several chemically unrelated substances produce similar double lead peaks and faster atomisation for not only lead, but also other volatile elements, *viz.*, zinc and cadmium. Naphthalene, used as a diluent for directly fired biological solids,[2] has been shown to affect the coincidence of the peak with that of an aqueous standard, making for inaccurate calibrations. Alkaline earth metals in the same matrix, particularly calcium, are known to affect lead appearance times and peak geometries.[1,4] The addition of ascorbic acid to these solutions produces an earlier atomised peak. Powdered graphite[5] has proved to be a benefit in vaporising inorganic solids, both enhancing and clarifying absorption signals. The furnace tube itself loses sufficient carbon with continued use to roughen the walls severely and a more intimate contact with the sample results.

Rapidly atomised elements, such as lead, present considerable problems in the attainment of accurate peak geometries. If the amplifier - recorder system operates at too narrow a band width (large time constant) a distorted record results. In particular, peak heights are severely shortened at higher atom concentrations. An earlier atomised maximum, such as is obtained with the addition of ascorbic acid, is likely to be more severely truncated than the following peak.

When twin peaks appear, analytical calibration becomes difficult, especially if the working standard does not produce an identical lead trace. Peak heights can be used only if the ratio of the twin peaks is controlled in both the sample and standard; that is, the charring of each must realise similar ratios of the two oxides. This situation is extremely difficult to achieve. The measurement of over-all peak areas is an obvious choice for an alternative

but there is evidence from the study of solutions of orchard leaves[2] that inaccurate results are still obtained if the aqueous standards do not produce traces that coincide absolutely with those from sample vaporisations.

The differences that can exist between orthorhombic massicot and tetragonal litharge residues, particularly with respect to the kinetics of the vaporisation - dissociation processes, have yet to be determined. The evidence suggests, however, that the former contributes to an earlier or more rapidly produced elemental lead vapour. Further, it seems certain that many organic matrix elements, carbon itself, competition with other refractory metals and some covalent mineral acids modify the charring processes such that different proportions of this more volatile form of lead(II) oxide are produced, perhaps through the propagation of H– or CH– intermediates.

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# Book Reviews

CONTAMINATION CONTROL IN TRACE ELEMENT ANALYSIS. By MORRIS ZIEF and JAMES W. MITCHELL. *Chemical Analysis, Volume* 47. Pp. xvi + 262. New York, London, Sydney and Toronto: John Wiley. 1976. Price £17.35; $28.

This excellent book brings together much of the scattered literature on the problems of contamination in trace element analysis. In addition the authors, who are both actively concerned with the subject (Zief as Principal Scientist for Purification, Baker Chemical Company, and Mitchell is with Bell Telephone Laboratories), describe in detail the manner in which they have overcome the problems in their own fields of work. Although there is some overlap with "Ultrapurity," co-edited by one of the authors (M. Zief and R. Speights, Marcel Dekker, New York, 1972), this does not detract from the value of the book as it is complementary rather than competitive. The contents can be broadly divided into two parts, contamination and methods for determining ultratrace elements. The first part deals comprehensively with apparatus, laboratory design, materials, purification of reagents and contamination control, while the second, apart from a detailed section on neutron-activation analysis, gives a rather cursory description of other analytical techniques such as X-ray fluorescence, spark-source mass spectrometry, emission spectroscopy and atomic-absorption and atomic-fluorescence spectroscopy. The layout of the book, and the rather short index, often will make it difficult for a worker to solve his particular contamination problem, although it may be described. Thus, although there is a section on the ashing of samples (p. 164), useful information on volatilisation losses of elements is given under neutron activation (p. 199, not indexed). The reviewer also has some reservations on the authors' use of some plastics, particularly poly(vinyl chloride). Although it is stated (p. 84) that solutions in contact with PVC may become contaminated with lead, titanium, zinc, tin, iron, magnesium and other cations from the additives used in its manufacture (but surprisingly no mention is made of cadmium), on p. 64 PVC gloves are suggested as affording the best protection for the analyst, and on p. 56 that stainless-steel sinks be protected with PVC linings. Similarly with polyethylene, although it is stated that for applications in trace analysis the use of the conventional low-density variety is preferable to that of the high-density (metal-catalysed) type, on p. 70 the latter is recommended for bottles for the storage of liquids. Although more emphasis might have been given to the necessity for the analyst to consider as suspect all of the materials and reagents he employs, the book is full of valuable information and fills a major gap in the literature on trace analysis. The reviewer regards it as essential for anyone engaged in this field of work.

R. O. SCOTT

THE QUALITY CONTROL OF MEDICINES. Edited by P. B. DEASY and R. F. TIMONEY. *Proceedings of the 35th International Congress of Pharmaceutical Sciences, Dublin* 1975. Pp. xiv + 398. Amsterdam, New York and Oxford: Elsevier Scientific Publishing Company. 1976. Price Dfl100; $38.50.

A book compiling the thoughts and experience of 32 pharmaceutical authoritarians at the end of the third quarter of this century must today be sensational. The book is superbly supported by 395 references, which lead the reader further into the specialised topics of physico-chemical methods and bioavailability. This is a collection of reviews echoing across the world the theme of the 35th International Congress of Pharmaceutical Sciences, "The Basis for the Quality Control of Medicines." The last three decades have seen the development of a range of antibiotics, starting with the sulphonamides and penicillin, and proliferating into the modern pharmaceutical industry, requiring national standards for safety and mutual recognition of its products internationally. Few chemicals are more exhaustively analysed than drugs, and the reviews show how the challenge of society through its regulatory bodies is met. Physico-chemical methods truly dominate the text, but the claim to have replaced the classical chemical methods is less obvious, on reading current pharmacopoeias. It is noted with satisfaction that the industry, by using the automatic analysis described, undertakes large numbers of on-line routine tests, thus leaving the trained analyst for more appropriate tasks. Hopes are raised on automation of microbiological assays using the degree of turbidity or evolution of carbon dioxide.

Chromatography, the analytical key to purification, is given justifiably high prominence. The full scope of thin-layer chromatography is documented, but the problems of quantification and

elution of the drug substance from the thin-layer plate remain unsolved, and it is left for high-performance liquid chromatography (HPLC) to perform the task. Some useful new information on HPLC is given: the variable wavelength ultraviolet detector meets the general need of the pharmaceutical analyst; the best solvents are often alcohols; the best columns are usually micro-particles of alumina or silica, often surface-bonded with octadecyl groups. Although one reads that gas chromatography is still the more sensitive method for detecting drug substances, this statement could require modification when fluorescence detectors are fully developed. Much value is placed on mass spectrometry in combination with gas chromatography—a technique suitable for very small samples, and giving identification after separation for metabolites of higher relative molecular mass than the drug substance. It is also interesting to learn that experiments have been carried out to discover relationships between fragmentation and pharmacological action.

At present there are marketed many highly potent drugs, such as the steroids, and the cardiac glucosides, which are poorly soluble in water. The effect of a change in the brand of digoxin tablets on the blood serum levels of 19 patients, we are told, was reported on the front page of the *New York Times*. The inadequacy of testing formulations of this nature solely on the basis of amount and impurities is graphically illustrated, supporting a current need for proof of bio-equivalence. Here one reads of confrontation between two regulatory authorities, the Food and Drug Administration and the United States Pharmacopeia, the former favouring *in vivo* and the latter the more convenient *in vitro* testing. Hope is expressed that *in vitro* methods, given sufficient funding for correlative studies and acceptance of the results by public review, could result in the pharmacopoeia principle gaining a higher level of authority.

The text highlights all modern methods of analysis. Fluorescence spectroscopy, its theory and application supported by six tables giving sensitivities in the microgram per kilogram range, is shown to advantage. Nuclear magnetic resonance spectroscopy, on the other hand, is dismissed on its value to describe the structure of a compound in a nearly complete way. The 1975 Congress in Dublin, where 1 700 people met to hear the contributors, will be remembered for the introduction of newer aspects of the quality control of drugs, the details of which are well presented in this historic publication.

E. W. HAMMOND

THE CHEMICAL ANALYSIS OF FOODS. Seventh Edition. By DAVID PEARSON. Pp. xii + 575. Edinburgh, London and New York: Churchill Livingstone. Distributed in the USA by Longman Inc., New York. 1976. Price £13.50.

The previous edition of this book was reviewed by A. W. Hubbard in 1971 (*Analyst*, 1971, **96**, 680). As the work is now firmly established as an indispensable text-book on food analysis, the current reviewer will concentrate on the changes introduced in the latest revision.

The basic format of the book remains essentially unaltered. Individual chapters consider proximate analysis, additives and contaminants, and then various groups of food commodities such as sugar and preserves, fruit and vegetable products, cereals and flour, starch products, beverages and chocolate, herbs and spices, fermentation products, meat and fish, dairy products and oils and fats. Each chapter follows a similar pattern in that the author first refers briefly to manufacturing processes and compositional requirements, including tables of typical analytical data for most products, and then summarises various food regulations. Relevant Food Standards Committee reports are discussed, together with a detailed account of the analytical examinations normally carried out to assess whether a sample satisfies the legal and compositional requirements. This section includes full experimental details of recommended methods, together with brief reference to alternative techniques and procedures. Guidance on the interpretation of analytical data is particularly valuable.

The principal changes introduced in the latest revision are the introduction of EEC legislation and the recommendations of the Codex Alimentarius Commission, reflecting the increasing influence of international standards on the work of the food chemist. Other changes incorporated include a switch to SI units and the transfer of literature references from the text to a composite list at the end of each chapter. The separate chapter on legislation has been removed, much of the information being added to the appropriate part of earlier chapters. There is also less emphasis on labelling requirements. Among subjects covered for the first time are the determination of cadmium, potassium, sodium, orotic acid and thiabendazole and the leaching of substances from packaging materials. Several recommended methods have been replaced by more up-to-date

procedures. As the Seventh Edition is about 30 pages shorter than the previous edition, inevitably some material has been discarded. In the chapter on starch products, micrographs of starch from 24 different sources have been reduced to 9, but the remaining pictures are larger and clearer.

As the title suggests, this book is concerned with chemical methods of food analysis. However, only brief reference is made to modern techniques such as atomic-absorption spectrophotometry and gas - liquid chromatography, while enzymatic methods, ion-selective electrodes and high-performance liquid chromatography receive no mention. One suggestion for further editions is the provision of "additional material for further study" at the end of each chapter. Such key references would require very little extra space and would make the work even more comprehensive as an encyclopaedia of food analysis. Ten appendices cover extracts from food regulations, energy values, SI units and various volumetric factors and spectrophotometric data. Selected relative atomic masses and logarithms are also included. Some of these data are, perhaps, more appropriate to a general text-book on quantitative analytical chemistry than to a book on food chemistry, particularly when space is restricted. A comprehensive index and list of abbreviations is also provided.

The book is well produced with clear type and diagrams; very few typographical errors were detected. It is undoubtedly very good value for anyone who has no copy of the Sixth Edition. The emphasis on classical rather than modern techniques may make the volume less attractive to someone who already has the earlier edition. N. T. CROSBY

TRANSPORT PROPERTIES OF IONIC LIQUIDS. By JAMES L. COPELAND. Pp. viii + 76. New York, London and Paris: Gordon and Breach Science Publishers. 1974. Price £5.

This volume will have a rather limited market but within that market it is likely to be in brisk demand. It is addressed to those engaged, or about to be engaged, in fundamental work in molten salt media. The author has felt the need for a compact survey of the state of the theoretical art in the chaotic and diffuse field of mass transport in ionic liquids. Eschewing what would be a multi-volume work embracing a complete review, he has compressed 20 years of research into 64 pages of text and 105 references, including a modest four of his own papers. Classifying emphasis into macro- and microscopic and attitude into pragmatic and fundamentalist, the vantage point of approach chosen is that of a chemist concerned with adjustable constants in which molecular interpretations are important, and in which inspired intuition in relating macroscopic properties to likely molecular characteristics is of great value.

General phenomenological concepts and definitions are first laid out under the headings of viscosity, diffusion and electrical conductance, without sparing the mathematics. There follows a general discussion of ionic melts including tabulations of properties. The remainder, rather more than half, of the text is devoted to mechanisms and models of ionic liquid transport, in which the hole theory, the absolute rate theory, and free volume theories and the glassy state concept are passed in review, together with a short treatment of the high-temperature region and further remarks on viscosity. In a field in which there are nearly as many theories and models as there are workers, the author has successfully striven to write without bias, and has made a judicious selection of material. A clear and concise introduction to molten salt chemistry and a good departure point for deeper study is offered. Although the field may seem particularly rarefied pure research without visible application, it contains seeds which may grow into the rationalisation of industrial molten salt electrochemistry and electrometallurgy, which may prove of great commercial benefit in these technologies. E. BISHOP

THEORY AND APPLICATIONS OF MOLECULAR DIAMAGNETISM. Edited by L. N. MULAY and E. A. BOUDREAUX. Pp. xiv + 321. New York, London, Sydney and Toronto: John Wiley. 1976. Price £22.50; $39.

The magnetic behaviour of molecules is a subtle reflection of the underlying electronic structure. A variety of different effects can be enumerated, which can be broadly grouped into two categories, diamagnetic and paramagnetic behaviour. It is to the former phenomenon that the Editors of this book have directed their attention, in the hope that they can "dispel the notion that studies on diamagnetic systems are not particularly useful in unravelling chemical problems." In realising this hope, the Editors have been only partially successful. It is certainly true that a wealth of detailed information has been collected, collated, tabulated and analysed but it still

remains true that diamagnetism is an effect most intimately connected with the behaviour of electrons in molecules and, unfortunately, an effect which cannot be distinguished from other effects so as to be clearly and uniquely correlated with some structural feature or utilised in isolation for some analytical purpose. Even so, a detailed study of diamagnetic behaviour is of great interest to chemists for the information that it can provide on the electronic structure of molecules and, in particular, the anisotropic properties of bonds.

The book consists of four main chapters by various authors and a very useful fifth chapter on definitions of the terms and units used in magnetic studies. The chapters are Introduction, Theory, Chemical Applications and Recent Advances, with the third topic (by Prof. Haberditzl) occupying about two thirds of the book. It is unfortunate that nowhere is there any indication of the experimental techniques that are used to measure diamagnetism, merely a few references to other, presumably more practically oriented, works by the Editors. The chapter on the theoretical basis of magnetic behaviour is, while doubtless accurate, unrelieved by any perceptive shafts of illumination. The reader is left to pick his own way through the kets and the bras,the operators and the matrices, with no kindly analogy and no helpful word of explanation to speed him on his way. This is particularly unfortunate as there are considerable problems in trying to devise suitable incremental systems for the correlation of molar diamagnetic susceptibilities. A more lucid theoretical chapter would enable the reader to evaluate more easily the various schemes described in the third chapter. This is a wide-ranging chapter with a detailed compilation of data and theories from both organic and inorganic systems. Sweeping generalisations are not possible, but the reader is certainly presented with all the facts and their current interpretations, for what they are worth. Particularly interesting are, of course, the diamagnetic effects apparently connected with so-called ring currents. Here again data and theories are comprehensively and objectively presented and discussed. One of the topics discussed in the last chapter is the bonding in organometallic compounds; here diamagnetic data has been used to select which of the many theoretical models for bonding in ferrocene is correct.

This book is informative but uninspired, detailed but uncritical. As an up-to-date compilation of data it is very good, but a person new to the field would find it heavy going. D. S. URCH

TOPICS IN ENZYME AND FERMENTATION BIOTECHNOLOGY 1. Edited by ALAN WISEMAN. Pp. 191. Chichester: Ellis Horwood. Distributed by John Wiley in Australia, New Zealand, South-East Asia, Canada, Europe and Africa, and by Halsted Press in the USA, South America and the rest of the World. 1977. Price £10.50; $19.95.

In the year that will see the first large-scale production in the UK of synthetic invert sugar from glucose, it is appropriate to welcome this splendid first volume that deals so admirably with the industrial use of glucose isomerase. The chapter on this enzyme is written by Dr. C. Bucke of Tate and Lyle Ltd. Other chapters are concerned with enzyme synthesis in continuous culture, aeration of fungal culture media and the foam separation of biological fluids. Drug synthesis using enzymes is covered in chapters on the penicillins, cephalosporins and the drug applications of microbial cytochromes. Apart from the editorial introduction, the book is completed by a useful chapter on patenting developments with micro-organisms.

Mr. M. Grylls of Distillers Company provides a lot of valuable advice on the law regarding patents, both in obtaining them and in opposing them. It is not generally appreciated that an invention is regarded as *novel* provided it has not been *published* or *used* within the UK before the Patent Application is lodged.

This first volume is nicely balanced between improving methods of microbial culture and using the products obtained therefrom. It is skilfully presented, meticulously edited and easy to read. It is highly recommended for chemists, biochemists and chemical engineers as a reference book written by experts at the forefront of their field. S. A. BARKER

ANALYSIS OF ESSENTIAL OILS BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY. By YOSHIRO MASADA. Pp. xiv + 334. New York, London, Sydney and Toronto: Halsted Press. 1976. Price £28.90; $49.70.

The book is divided into two parts, of which the first contains separate monographs on individual essential oils. The second, much shorter, part appears to contain details of instrumental techniques, but as it is written in Japanese, a more precise description is very difficult. The second part is followed by a short appendix, relating to individual essential oil components, and an

alphabetical index. The essential oils are dealt with in botanical groups and the book is preceded by an index giving the botanical groups and the individual oils within the groups.

Each essential oil is the subject of a separate monograph containing a brief description of the source, origins and uses and a table of the physical and chemical constants. The main chemical components are listed and one or more mass spectra of the the major components are shown diagrammatically. Also included is a gas chromatogram obtained from a temperature programmed capillary column of Carbowax 20M. Each of these chromatograms has been run under a standard set of conditions but there is some variation of the relative retention times for the same compounds in different oils and different chromatograms. Each monograph carries an informative list of references.

The extent of the information in each monograph varies and some of them carry further gas chromatographic evidence and identification of components after physical separation. In a volume of only 300 pages or so, it is impossible to include all of the known essential oils and there are some omissions. The book is particularly lacking in details of similar oils from different geographical sources. However, it is very informative and completely free from "padding" or repetition. It is refreshing to see so much evidence produced by the author himself and the book leaves us with a tantalising glimpse of Japanese analytical expertise illuminated only by the diagrams.

A. M. HUMPHREY

TABELLEN ZUR STRUCTURAUFKLÄRUNG ORGANISCHER VERBINDUNGEN MIT SPECKTROSKOPISCHEN METHODEN. By E. PRETSCH, T. CLERK, J. SEIBL and W. SIMON. *Anleitung für die chemische Laboratoriumspraxis, Band* XV. Pp. x + 307. Berlin, Heidelberg and New York: Springer-Verlag, 1976.

This reference text contains tabulated information mainly on $^{13}C$ and $^{1}H$ NMR shifts for a wide range of organic compound types, IR bands for an equally wide range of functional groups, mass spectral peaks for molecular fragments and isotopes and characteristic UV - visible absorption wavelengths and spectra. The bulk of the data is assembled in chapters devoted to each spectroscopic technique, but there is also a section in which the data from all the techniques are collected together for each type of compound. This dual arrangement is very useful and will greatly assist the correlation or interpretation of spectral information with respect to molecular structure and enable chemists more rapidly to reach structural conclusions. The material is clearly reproduced from typescript. The German text should present few problems to the non-linguist as most of the material is numerical, with chemical names and formulae, and the amount of discussion is kept to a minimum.

A. TOWNSHEND

ESTUARINE CHEMISTRY. Edited by J. D. BURTON and P. S. LISS. Pp. xii + 229. London, New York and San Francisco: Academic Press. 1976. Price £8; $17.50.

The estuarine environment is a critical area of physicochemical interaction which both controls the global input of elements to the open ocean and also determines the fate of pollutants in waters, sediments and organisms in areas often close to human habitation. The chemical processes which occur in this transitional zone of extreme property gradients are necessarily complex and are, as yet, only partly defined. Despite the increasing research effort which, largely through environmental pressures, has recently been focused on estuarine (and coastal marine) chemistry, there has been no text available to reflect or document that trend.

This book, therefore, represents the first response to a major requirement for a review text and, as such, is a valuable asset. It is, in essence, a compilation of seven papers, somewhat extended and revised, presented at a 1-day conference in May, 1974. As a result, the book falls short of the comprehensiveness implied by its title, firstly because it is now 3 years behind the advancing frontiers and, more important, because it contains a slightly fragmented mixture of selected review and research articles. Estuarine chemistry merits a volume of considerably greater size and content than this one, with many of the present chapters sub-divisible into more comprehensive units and additional topics included, for example, descriptions of the physical and geological characteristics which influence many estuarine interactions.

The principal criticism, therefore, concerns the disparity in scale between this book and the demand for it, rather than reflecting on the inherent quality of its contributions, for it contains well written chapters by prominent research workers on basic chemical processes, sedimentation, the behaviour characteristics of organic, dissolved and metallic species and on radioactive tracers in estuarine systems. The final chapter differs markedly from the main text, being essentially a

research paper dealing specifically with the Clyde estuary. While this contribution would have little place in a review volume it does require the reader to re-orientate from the general to the specific, a necessary transition for anyone working in the environmental sciences.

In conclusion, this book is essential reading for all concerned with estuarine systems. If it is less comprehensive than desired there are plentiful references for further reading. Curiously, the contribution by E. K. Duursma is valuable for an inverse reason, namely that the author and co-workers have pioneered definitive studies of element speciation and transfer via radioactive tracer methods but invariably have published their results in journals of limited availability in the UK. The book does, in fact, have a strong British bias but its appeal should be universal, in line with a growing suspicion that while the UK may not always lead in marine science research, it invariably provides the best texts in that field.

M. S. BAXTER

DETERMINATION OF FOOD CARBOHYDRATES. D. A. T. SOUTHGATE. Pp. x + 178. London: Applied Science. 1976. Price £10.

The carbohydrates in food have been grouped from a nutritional point of view into those which are available to man by digestion and absorption and those which are unavailable and not digested in the human digestive tract by its endogenous secretions. The author uses this concept as a framework for his book but covers each group of carbohydrates, such as sugars, dextrins and starches (available) pectins, hemicelluloses and celluloses (unavailable), in detail. Non-structural, unavailable carbohydrates, found naturally or used as food additives (algal polysaccharides, gums, mucilages, etc.), are not forgotten. Specific groups of foods (milk, milk products, eggs, meat, fish, cereals, vegetables, fruits, beverages) are considered and selected methods of analysis of particular carbohydrates are given with adequate experimental details. A useful appendix contains relationships between specific gravity, degrees Brix and degrees Baumé of sugar solutions as well as factors for use with the Lane - Eynon and Munson and Walker methods.

The book reflects the vastly increased use of enzymes in analysis for the specific determination of a given food carbohydrate constituent and could have been taken further by pointing out the advantages of immobilised enzymes in analysis, whether presented as a tubular reactor, a column packing or as a component of an enzyme electrode. However, the solid groundwork is well covered except for inadequate information on automated methods.

The other major recent trend in analysis is to separate mixtures much more adequately before assaying them and the book deals briefly with ion-exchange, gas - liquid, paper and thin-layer chromatography. High-performance liquid chromatography (HPLC), the fastest evolving technique in this area of analysis, is strangely absent. While polarimetric methods of sugar analysis are crude, convenient methods and excellent for comparative purposes, many is the customer who has been duped when the sugar contained polysaccharide or other impurities. It is here that HPLC should have been advocated. Those entering the area of food technology will find this a useful book and excellent guide. Those already in the field will do well to read it and ponder on the thought that for food carbohydrates, separation with analysis is the safest procedure and that enzyme electrodes will figure largely in a similar book written 5 years hence.

S. A. BARKER

HPTLC: HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY. Edited by A. ZLATKIS and R. E. KAISER. *Journal of Chromatography Library, Volume* 9. Pp. xiv + 240. Amsterdam, Oxford and New York: Elsevier. Bad Dürkheim: Institute of Chromatography. 1977. Price Dfl110; $43.95.

This is a nine-chapter work by six contributing authors, namely, J. Blome, H. Halpaap (with J. Ripphahn), U. B. Hezel, D. Jaenchen and R. E. Kaiser. Although presented in the cloak of the slick initials HPTLC, the book is essentially a commercially based thin-layer chromatography work, its contents being devoted to simplified theory, linear and circular techniques, the U-chamber, dosage and development techniques, reproducibility and quantitative aspects.

A great deal of practical information is provided but what a pity that the editing is of such poor quality. Thus, the generally well drawn figures are erratically numbered and in some chapters are not numbered at all, section headings listed in the Contents are frequently not given in the text and there are typographical errors. It was illuminating to read that "*two centuries* passed before Tswett's column chromatography was re-discovered" (p. 51)!

The prospective purchaser is advised to examine this book carefully before deciding to spend around £25.

J. D. R. THOMAS

## Identification and Determination of Alkyl Xanthate Mixtures

A procedure for the identification and determination of alkyl xanthate mixtures based on high-performance liquid chromatography is described. The procedure is based on the oxidation of alkyl xanthates to dialkyldixanthogens. The relative retention times of the dialkyldixanthogens are given. The response of the dialkyldixanthogens is approximately proportional to the product of the concentrations of the alkyl xanthates from which they are formed. The alkyl xanthates that were studied were the ethyl, 1-propyl, 2-propyl, 2-methyl-1-propyl, 1-butyl, 2-butyl and 1-pentyl xanthates. On-column derivatisation, *i.e.*, oxidation, was found to occur under the appropriate conditions and its characteristics are discussed.

*Keywords*: *Alkyl xanthate determination*; *dialkyldixanthogens*; *alkyl xanthate oxidation*; *high-performance liquid chromatography*; *mineral processing*

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*Analyst*, 1977, **102**, 519–524.

## Fluorescent Properties of Some Butyrophenones

A spectrofluorimetric investigation of therapeutically used butyrophenones (neuroleptic drugs) is described. These substances, under certain conditions, emit fluorescent light on exposure to ultraviolet radiation.

The first part deals with the fluorescence of the butyrophenones in solution; the qualitative and quantitative aspects of the phenomenon depend on the solvent. Benperidol, droperidol, azaperone and fluanisone exhibit a distinct signal, caused by the heterocyclic substitution of the 4-aminobutyric acid chain. Qualitative tests as well as quantitative determinations were carried out, the latter requiring preliminary removal of interfering substances that absorb ultraviolet radiation. Haloperidol, bromoperidol, trifluperidol and pipamperone exhibit a weak signal.

The influence of acidity and nature of the solvent on the fluorescence was examined and the optimum pH range and the appropriate wavelengths of excitation and emission were established for the determination of each species; limits of detection are reported. Adjuvants that absorb ultraviolet radiation within the range of excitation or emission spectra need to be extracted.

The action of strong acids on the fluorescence of trifluperidol and pipamperone is discussed. Azaperone fluoresces intensely in acidic medium; direct and sensitive determinations can be performed with a limit of detection of $10^{-2}$ $\mu$g ml$^{-1}$. The drug can be made visible on chromatographic plates by its fluorescence in acidic medium.

*Keywords*: *Butyrophenones*; *drugs*; *spectrofluorimetry*

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*Analyst*, 1977, **102**, 525–530.

## Optoacoustic Spectrometry of Solid Materials: Effect of the Filler Gas on the Observed Signal

*Short Paper*

*Keywords*: *Optoacoustic spectrometry*; *filler gas*; *solid samples*; *xenon power spectrum*; *gas molecular diameter*

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*Analyst*, 1977, **102**, 531–534.

## Effects of Emulsifiers and Stabilisers on the Determination of Salt in Meat Products

*Short Paper*

*Keywords*: *Salt determination*; *meat products*; *emulsifiers*; *stabilisers*

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*Analyst*, 1977, **102**, 534–537.

## Enhancement of the Sensitivity of the Cold-vapour Atomic-absorption Spectrophotometric Method Towards Mercury by Using a Sintered-glass Bubbler and a Magnetic Stirrer

*Short Paper*

*Keywords*: *Mercury determination*; *cold-vapour atomic-absorption spectrophotometry*; *food analysis*

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*Analyst*, 1977, **102**, 537–540.

## Spectrophotometric Determination of Malonate with 4-Hydroxybenzaldehyde

*Short Paper*

*Keywords*: *Malonate determination*; *4-hydroxybenzaldehyde*; *spectrophotometry*

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*Analyst*, 1977, **102**, 540–542.

## Double Peaks in the Atomic-absorption Determination of Lead Using Electrothermal Atomisation

*Short Paper*

*Keywords*: *Atomic-absorption spectrophotometry*; *electrothermal atomisation*; *lead determination*; *multiple peaks*

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