The ANALYST

The Journal of the Society for Analytical Chemistry

A monthly International Publication
dealing with all branches of
Analytical Chemistry

Published by the
SOCIETY FOR ANALYTICAL CHEMISTRY

Volume 99

No. 1181, Pages 457-536

August, 1974

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THE ANALYST

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Published by The Society for Analytical Chemistry
Distributed by The Chemical Society, Publications Sales Office,
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Volume 99, No. 1181 August, 1974

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Summaries of Papers in this Issue

The Determination of Trace Amounts of Lead in Steel by Anodic Stripping Voltammetry

A procedure is described for the direct determination of lead in steel samples by anodic stripping voltammetry. The method is both simple and rapid, about 1 hour being needed to complete an analysis. No chemical operations are required other than sample dissolution, which minimises the risk of contamination or losses of lead. Interferences to the method are few, only copper (more than 0·9 per cent.) and molybdenum (more than 0·1 per cent.) being of any significance.

The detection limit of the method was calculated to be 0·0001 per cent. of lead and the accuracy and precision have been established by the analysis of a wide range of standard steel samples.

B. METTERS and B. G. COOKSEY

Department of Pure and Applied Chemistry, University of Strathclyde, Cathedral Street, Glasgow, G1 1XL.

Analyst, 1974, **99**, 457–468.

A Modification to the Extraction - Atomic-absorption Method for the Determination of Antimony, Bismuth, Lead and Tin

It has been shown that the use of organometallic compounds for the calibration procedure used in the extraction - atomic-absorption determination of antimony, bismuth, lead and tin in metallurgical materials leads to erroneous results at low concentration levels. Results are presented, which demonstrate that the technique is satisfactory when calibrated with solutions that have been taken through the extraction procedure.

K. THORNTON

Henry Wiggin and Co. Ltd., Holmer Road, Hereford.

and KEITH E. BURKE

The International Nickel Company, Inc., Paul D. Merica Research Laboratory, Sterling Forest, Suffern, New York 10901, U.S.A.

Analyst, 1974, **99**, 469–470.

Spectrophotometric Determination of Magnesium in Tobacco Leaves with Eriochrome Black B

A spectrophotometric method for the determination of magnesium in tobacco leaves with Eriochrome black B is described. After destruction of the tobacco leaves with a mixture of nitric, perchloric and sulphuric acids, the magnesium is separated from interfering ions by ion-exchange chromatography on Dowex 50W-X12, 100 to 200 mesh, and determined spectrophotometrically after coupling it with Eriochrome black B. The accuracy of the method was tested against a reference material.

G. SLEGERS and A. CLAEYS

Laboratory of Analytical Chemistry, Faculty of Pharmaceutical Sciences, State University of Ghent, 9000 Ghent, Belgium.

Analyst, 1974, **99**, 471–475.

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The Spectrophotometric Determination of Ampicillin and Cloxacillin in Combined Injections

A method is described for the determination of ampicillin and cloxacillin in injections. Ampicillin is determined by an absorbance difference technique based on the higher absorbance of ampicillin at 268 nm in a solution at pH 5 than in one at pH 9. Cloxacillin is determined by measurement of the absorbance at 275 nm and the application of a small correction for the absorbance of ampicillin. The accuracy, precision and specificity of the method are discussed. The analytical results obtained for commercial samples of ampicillin - cloxacillin (1 + 1 and 2 + 1) mixtures are compared with those obtained by microbiological assay.

A. G. DAVIDSON and J. B. STENLAKE

Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow, G1 1XW

Analyst, 1974, **99**, 476–481.

Colorimetric Determination of Piperazine in Pharmaceutical Formulations

Piperazine can be satisfactorily determined in pharmaceutical preparations or formulations such as effervescent granules and elixirs containing hexamine, colchicine, atropine sulphate, sodium benzoate, lithium benzoate, lithium citrate, sodium citrate, sodium hydrogen carbonate, tartaric acid, citric acid, lactose, sucrose and Tinct. ammi visnaga. The diluted sample solution is treated with a 0·6 per cent. aqueous 1,2-naphthoquinone-4-sulphonate solution in the presence of acetate - citrate buffer at pH 7·5. The temperature of the reaction should be between 10 and 15 °C and the colour produced is measured at 490 nm.

YEHIA M. DESSOUKY

Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

and SAAD A. ISMAIEL

Research Department, Société Misr pour l'Industrie Pharmaceutique, 92 El Mataria Street, Post El Zeitoun, Cairo, Egypt.

Analyst, 1974, **99**, 482–486.

Colorimetric Determination of Antazoline in Some Pharmaceutical Preparations with Sodium Nitrite

A colorimetric method for the determination of antazoline in pharmaceutical preparations with sodium nitrite has been developed. The method involves treatment of a cooled and acidified dilute aqueous solution of the sample with sodium nitrite. The yellow colour produced is stabilised by the addition of propan-2-ol or ethanol and the absorbance then measured at 410 nm.

Naphazoline, tolazoline, clemizole, diphenhydramine, chlorpheniramine, ephedrine, cetrimide, benzalkonium chloride and zinc salts, even if present in amounts ten times greater than that of antazoline, do not interfere.

M. M. AMER, M. S. TAWAKKOL

Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

and S. A. ISMAIEL

Research Department, Société Misr pour l'Industrie Pharmaceutique, 92 El Mataria Street, Post El Zeitoun, Cairo, Egypt.

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The Determination of Trace Amounts of Lead in Steel by Anodic Stripping Voltammetry

BY B. METTERS AND B. G. COOKSEY

(Department of Pure and Applied Chemistry, University of Strathclyde, Cathedral Street, Glasgow, G1 1XL)

A procedure is described for the direct determination of lead in steel samples by anodic stripping voltammetry. The method is both simple and rapid, about 1 hour being needed to complete an analysis. No chemical operations are required other than sample dissolution, which minimises the risk of contamination or losses of lead. Interferences to the method are few, only copper (more than 0·9 per cent.) and molybdenum (more than 0·1 per cent.) being of any significance.

The detection limit of the method was calculated to be 0·0001 per cent. of lead and the accuracy and precision have been established by the analysis of a wide range of standard steel samples.

POLAROGRAPHIC techniques, in particular anodic stripping voltammetry, are well suited to the determination of lead in a wide variety of materials. In recent years, however, d.c. polarography has seldom been used as an analytical tool for trace-metal analysis following the advent of the more convenient technique of atomic-absorption spectroscopy. This latter technique and spectrophotometry[1,2] are the main methods currently employed for determining lead in steel samples. Both techniques are limited to determinations of lead in the range 0·1 to 0·01 per cent. in solutions containing 1·0 g of steel per 100 ml and solvent extraction[3–5] is required for the determination of lower levels. The solvent-extraction step is necessary either to provide a sufficiently concentrated solution for the final measurement or to remove interfering ions, particularly iron(III) and copper(II), as described by Hofton and Hubbard.[5]

Such procedures are extremely lengthy and frequently involve more than one extraction step as well as the necessary washing of the solvent. In addition, determinations of lead at concentrations below 0·002 per cent. in the steel are unreliable because at this level, losses of lead in the extraction steps, and contamination from the large surface area of the glassware and variety of chemicals used, become significant. Therefore, any direct procedure in which solvent extraction is eliminated would be advantageous. Such a method, however, must be extremely sensitive and relatively free from interferences. Anodic stripping voltammetry partially satisfies both of these demands as it combines the selectivity of polarography with great sensitivity (*e.g.*, lead can be determined down to a concentration of 1×10^{-9} M in pure water).

Many elements have been determined by this method and a review of the literature has been given.[6] Initially, the technique was used only for the determination of zinc, cadmium, lead and copper in water samples[7–9] because of the relatively poor selectivity caused either by overlap of stripping peaks or by a large background current that resulted from a high concentration of components in the sample solution, *e.g.*, acids and iron(III) in solutions of steel samples.

These difficulties have been overcome by several workers,[10–12] who included a "medium exchange" step in the method. The electrolytic concentration step is carried out in the usual way in the sample solution, but the stripping or oxidation procedure is carried out in an alternative medium, usually a pure electrolyte, *e.g.*, potassium chloride, perchloric acid, etc.

In this way, large residual currents are eliminated from the stripping polarogram with little or no effect on the oxidation currents due to the metal ion under test, and by careful choice of this medium a good separation of oxidation peaks can be obtained.

By using medium exchange, complex solutions can be analysed and Ariel, Eisner and Gottesfield[10] applied the technique to the determination of copper in Dead Sea brine and

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of copper in the presence of manganese and bismuth. This work was followed by application of a method for determining copper in steel samples.[12] Phillips and Shain[11] also used medium exchange in the determination of tin in steel samples, but anodic stripping voltammetry does not appear to have been used for a similar determination of lead.

The method proposed in this paper is direct, rapid and relatively simple, medium exchange being necessary only when molybdenum and fairly large amounts of copper are present. Interferences are few and the method has been applied to the determination of lead in a wide variety of steel and cast iron samples with a detection limit of 1×10^{-4} per cent. of lead.

Experimental

Apparatus—

The instrument used was a Radiometer, Copenhagen, PO4 polarograph. The experiments were carried out in a water-jacketed 40-ml glass electrolysis cell at 25 °C with a three-electrode system that consisted of a saturated calomel reference electrode, a platinum-wire counter electrode and a hanging mercury drop electrode (H.M.D.E.). The solution was stirred by means of a rotating glass paddle stirrer operating at 600 r.p.m. and was de-oxygenated with a stream of nitrogen from an inlet tube. The cell arrangement is shown in Fig. 1.

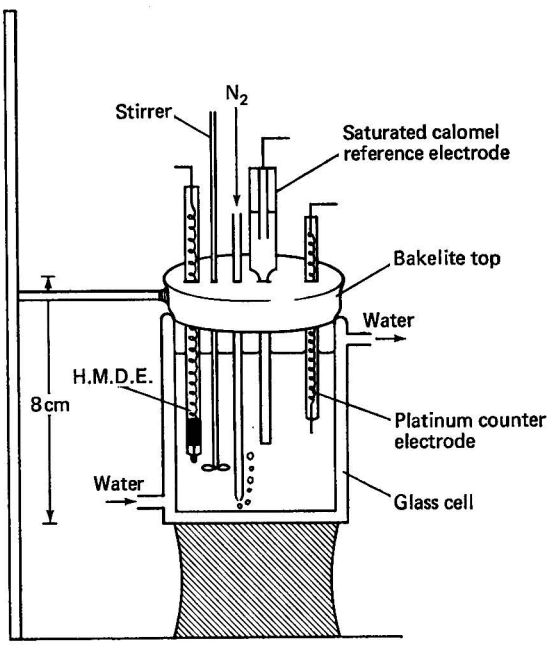

Fig. 1. Electrode assembly (H.M.D.E. denotes hanging mercury drop electrode)

The two leads from the polarograph were connected to the three electrodes via the specially constructed electronic system represented in Fig. 2. (This electronic unit is not required if a polarograph suitable for use with three electrodes is used.)

Recommended method for the preparation of the hanging mercury drop electrode—

Seal about 2·0 cm of 22-gauge platinum wire into a 10-cm length of 7 mm o.d. soda-glass tube in a bunsen flame. Cut off the protruding wire, grind it flush with the glass on a suitable rotating grinder and polish the end of the electrode with successively finer grades of emery cloth. Then etch the tip of the electrode in hot aqua regia for several minutes and rinse it well with distilled water. In order to achieve electrical contact, partially fill

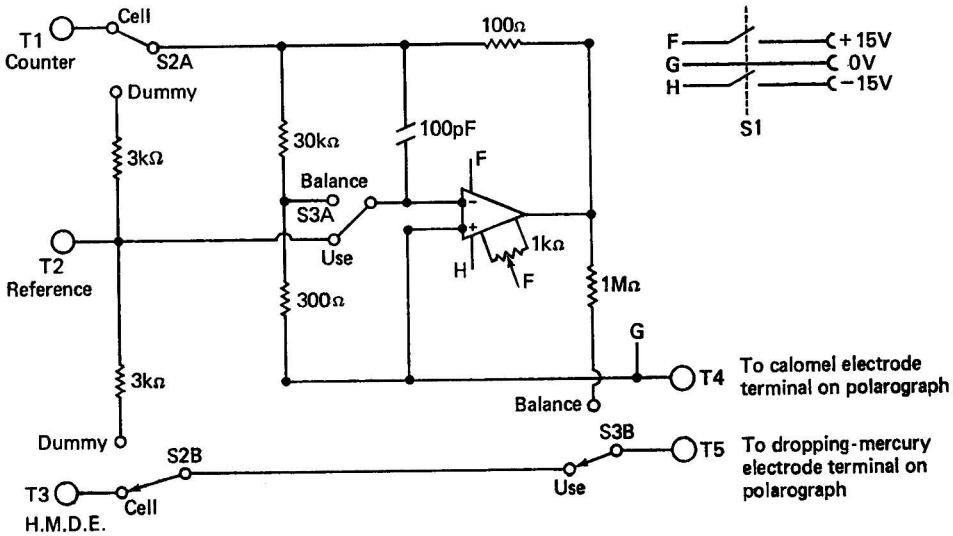

Fig. 2. Circuit diagram of the electronic unit for the conversion of a two-electrode polarograph to a three-electrode system. S1, on - off; S2, dummy - cell; and S3, balance - use

the tube with mercury and place a length of copper wire in the tube as shown in Fig. 3. The electrode is now ready to be electroplated with mercury.

ELECTROPLATING THE H.M.D.E.—

Place the H.M.D.E. and a platinum-wire counter electrode in a beaker containing 10 per cent. *V*/*V* nitric acid. Connect the two electrodes to a constant-current device and alternately anodise then cathodise the H.M.D.E. for 15 s at 25 mA, finishing with cathodic electrolysis for 15 s. Rinse the electrodes with distilled water and place them in a beaker containing concentrated mercury(II) nitrate solution that is 1 M in nitric acid. Now cathodise the H.M.D.E. for several minutes at 25 mA until the exposed platinum tip is completely covered with mercury. Rinse it well with distilled water, shaking off any excess of mercury globules that may have formed during the electrolysis.

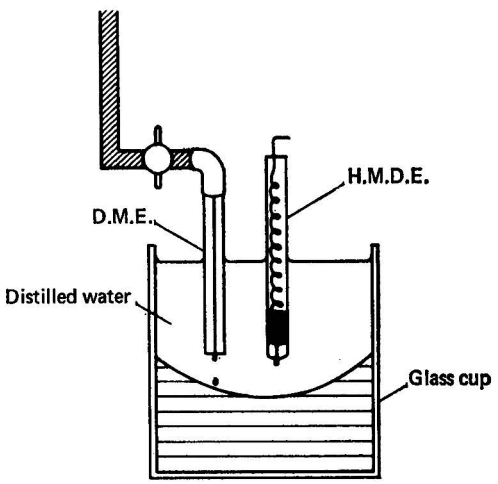

Fig. 3. The hanging mercury drop electrode (D.M.E. denotes dropping-mercury electrode)

The electrode should now be capable of picking up individual drops of mercury reproducibly delivered from a dropping-mercury electrode, as shown in Fig. 3. (If a constant-current device is not available, the electroplating procedure can be carried out in the electrolysis cell by using a three-electrode system, *i.e.*, H.M.D.E., platinum counter electrode and reference electrode. The H.M.D.E. should be cleaned with nitric acid, anodised and cathodised at $\pm 1{\cdot}5$ V *versus* S.C.E., then electroplated in the mercury(II) nitrate solution at $-1{\cdot}5$ V *versus* S.C.E.)

The electroplating procedure needs to be carried out only once a week provided the electrode is stored in distilled water when not in use. It is necessary, however, to use a fresh drop of mercury for each determination of lead. Throughout this work two drops of mercury were collected in the glass cup from a 72-cm head of mercury, which resulted in a single drop weighing approximately 11·0 mg.

Treatment of glassware in order to reduce adsorption—

The electrolysis cell, calibrated flasks and pipettes were thoroughly cleaned in dilute nitric acid and allowed to stand in distilled water for 24 hours. Following a thorough rinsing with distilled water, the flasks and electrolysis cell were treated with a 2 per cent. solution of dimethyldichlorosilane in carbon tetrachloride for 24 hours. (A commercial form of this solution, Repelcote, is available from Hopkin & Williams Ltd.) The apparatus was then rinsed with methanol and finally several times with distilled water. (For the analysis of steels a 150-ml PTFE beaker was used during the dissolution of the samples.)

Reagents—

All chemicals were of analytical-reagent grade purity and solutions of them were prepared with distilled water from an all-glass distillation apparatus[13] and stored in polythene bottles so as to minimise contamination. A stock $1{\cdot}0 \times 10^{-3}$ M solution of lead was prepared by dissolving analytical-reagent grade lead nitrate in distilled water, from which a standard $1{\cdot}0 \times 10^{-5}$ M solution was prepared daily. Oxygen-free nitrogen was used throughout this work for de-oxygenating test solutions.

Recommended method for the analysis of steel samples—

Procedure A—Weigh 1·0 g of steel sample into a PTFE beaker and add 10·0 ml of 40 per cent. nitric acid (for stainless-steel samples, add 5·0 ml of 60 per cent. perchloric acid); heat the mixture until the sample has dissolved, then dilute and filter the resulting solution into a 100-ml calibrated flask, wash the insoluble residue and make the combined filtrate and washings up to volume (solution 1).

To a 10-ml aliquot of solution 1, add 5·0 ml of 10 per cent. ascorbic acid solution and 1·0 ml of 0·1 M zinc nitrate solution and dilute the mixture to 100·0 ml in a calibrated flask (solution 2). Carefully rinse the electrolysis cell twice with a few millilitres of solution 2 and then fill the cell to within about 1 cm of the top of the cell (*i.e.*, about 30·0 ml). De-oxygenate the sample solution by bubbling nitrogen through the solution for 5 minutes and then raise the gas bubbler above the solution so as to allow nitrogen to flow over the surface of the solution throughout the remainder of the test in order to maintain an inert atmosphere. Catch a drop of mercury of suitable size on the H.M.D.E. (Fig. 3), place the latter in the cell and connect all electrodes to the relevant terminals.

Switch on the rotating glass paddle stirrer (600 r.p.m.) and apply a potential of $-0{\cdot}6$ V *versus* S.C.E. to the H.M.D.E., simultaneously starting a stop-watch in order to time the electrolysis. At the end of the required electrolysis time (15 minutes for steels containing 0·0001 to 0·002 per cent. of lead and 3 minutes for those containing 0·002 to 0·02 per cent. of lead), switch off the stirrer and allow 30 s for the solution to become quiescent. Now record the current - voltage stripping curves, using either of the procedures described below.

Procedure B (for steels containing less than 0·2 per cent. of copper and less than 0·02 per cent. of molybdenum)—Scan the voltage of the H.M.D.E. from $-0{\cdot}6$ to $+0{\cdot}4$ V *versus* S.C.E. at the rate of 0·4 V min^{-1}, recording the current - voltage curve. Measure the peak height at $-0{\cdot}3$ V *versus* S.C.E.

Procedure C (for steels containing 0·2 to 0·95 per cent. of copper and/or 0·02 to 0·10 per cent. of molybdenum)—Remove the electrolysis cell from under the electrode assembly, discard the test solution, refill the cell with a solution that is 5×10^{-3} M in sodium acetate and

1×10^{-3} M in Zn^{2+} and contains 0·5 per cent. of ascorbic acid, which has previously been de-oxygenated by bubbling nitrogen through it, and replace the cell under the electrodes (this operation should take not more than 10 s). Scan the voltage of the H.M.D.E. from −0·6 to +0·4 V *versus* S.C.E. at the rate of 0·4 V min^{-1}, recording the current - voltage curve.

Results and discussion

Calibration graphs were prepared for lead in acetate buffer medium but straight lines were not obtained with the use of either distilled or de-ionised water (Fig. 4). It was thought that the curvature was due to the existence of a lead complex, and all solutions were acidified in the hope that such a complex would not be formed in acidic media. Although this treatment had no effect, the addition of both acid and zinc ions did result in the production of straight-line calibration graphs (Fig. 4) (typical stripping peaks are shown in Fig. 5).

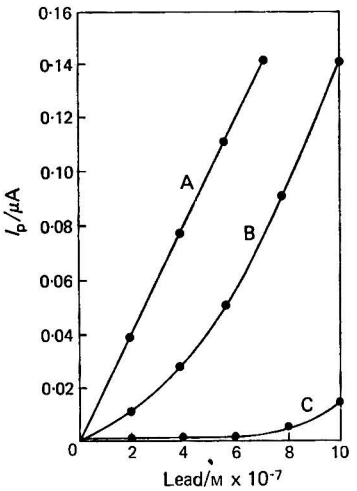

Fig. 4. Calibration for lead in pure aqueous solutions. T_d (electrolysis time), 3 minutes; E_e (electrolysis potential), −0·6 V *versus* S.C.E.; and V_s (voltage scan rate), 0·4 V min^{-1}. A, $HClO_4$ + Zn^{2+}; B, distilled water + CH_3COONa or $HClO_4$; and C, de-ionised water + CH_3COONa or $HClO_4$. I_p is peak current

This effect is not fully understood but there are two possible explanations: (*i*) in distilled water and certainly in de-ionised water, minute amounts of complexing agents are present, which are capable of forming strong complexes with lead even in acidic solution. The lead complex, once formed, is not reduced at the H.M.D.E. in the available potential range, and only when there is an excess of free lead ions in solution is a peak observed at −0·3 V *versus* S.C.E.

This result explains why the effect is more marked in de-ionised water, which contains additional complexing agents washed over from the ion-exchange resin during purification. It is assumed that zinc forms a stronger complex than lead with the impurity and that it is an effective releasing agent. Therefore, provided sufficient zinc is added to the test solutions, any lead present will exist as free ions and a straight-line calibration graph will be obtained.

(*ii*) The solutions contain a surface-active impurity, which is adsorbed on to the surface of the H.M.D.E. and thus prevents effective reduction of lead ions until a certain concentration of lead is reached. This inference could also explain the more marked effect in de-ionised water, which is more likely to contain organic material from the ion-exchange resin that may be adsorbed on to the H.M.D.E. In this instance it is assumed that the zinc ion is preferentially adsorbed on to the H.M.D.E. and does not prevent efficient electro-reduction of lead ions.

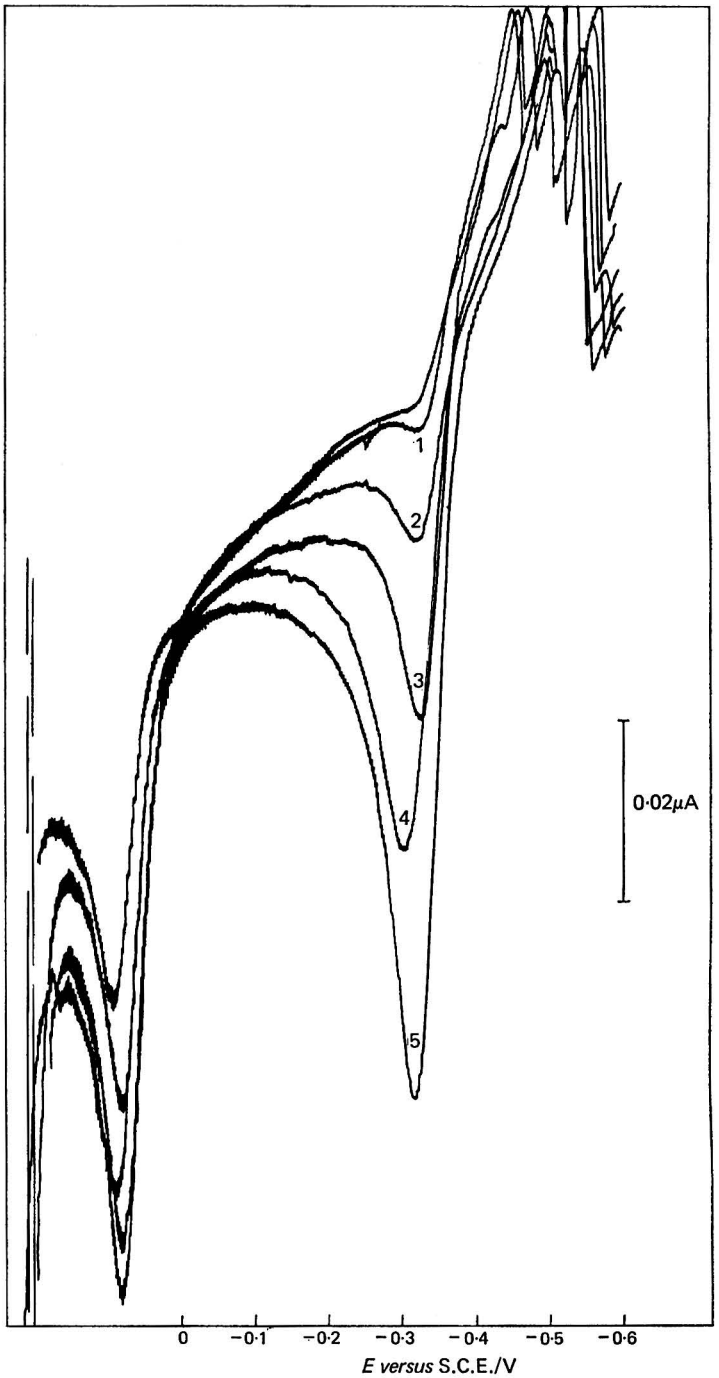

Fig. 5. Typical stripping peaks for lead. Electrolysis time, 15 minutes, electrolysis potential, −0·6 V *versus* S.C.E. and voltage scanning rate, 0·4 V min^{-1}. Solutions contained 1·0 g l^{-1} of iron (prepared from pure iron powder), 1·0 × 10^{-3} M of Zn^{2+} and 0·5 per cent. of ascorbic acid, together with the equivalent of lead, per cent.: 1, 0·0; 2, 0·0004; 3, 0·0008; 4, 0·0012; and 5, 0·0016; the peak at +0·1 V *versus* S.C.E. is due to copper contamination

In view of the above results, both acidification of and the addition of zinc ions to all solutions are recommended as a precautionary measure in the determination of lead.

CALIBRATION GRAPHS IN THE PRESENCE OF IRON—

Polarographic analysis of steel samples is not possible with solutions containing iron(III) because of the large cathodic residual current that begins at zero applied voltage from the reaction,

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

Iron(II), however, is not reduced at a mercury electrode until a potential of −1·4 V *versus* S.C.E. in acidic solution is reached and, therefore, the interference of iron(III) is easily overcome by chemical reduction with ascorbic acid. Calibration graphs for two ranges of lead concentration were obtained in solutions containing 1·0 g l^{-1} of iron to which ascorbic acid had been added [Fig. 6 (*a* and *b*)]. The results in Fig. 6 (*b*) are similar to those in Fig. 4 and it is apparent that the presence of iron and ascorbic acid has no effect on the electro-deposition or subsequent stripping of lead at the H.M.D.E.

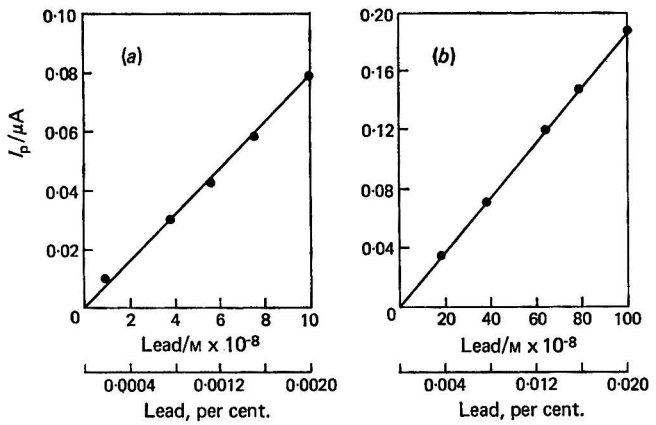

Fig. 6. Calibrations for lead in the presence of 1·0 g l^{-1} of iron. (*a*) Solutions contained 1·0 g l^{-1} of iron, 1·0 × 10^{-3} M of Zn^{2+} and 0·5 per cent. of ascorbic acid. Electrolysis time, 15 minutes. (*b*) Solutions as for (*a*). Electrolysis time, 3 minutes

INTERFERENCES—

The first step in most analytical methods is the preparation of a suitable sample solution and, in steel analysis, it is accomplished by the addition of an acid or a mixture of acids. The choice of acids in a procedure for the determination of lead by anodic stripping voltammetry is restricted to either nitric acid or perchloric acid. Hydrochloric acid cannot be used because in chloride media the stripping peak for lead is considerably less than that in other media[8] and the sensitivity is reduced owing to the merging of the lead peak with the oxidation current of mercury.[10] A similar effect has been observed in this laboratory when using sulphate media.

The complex composition of steel also indicates that many other metal ions will be present in the sample solution. Additions of manganese, cobalt, aluminium, barium and calcium up to a concentration of 1 × 10^{-3} M, and of nickel and chromium up to 5 × 10^{-3} M, to a solution that is 4 × 10^{-7} M in lead and contains 1·0 g l^{-1} of iron and 0·5 per cent. of ascorbic acid had no effect on the stripping peak for lead. The elements that would be expected to interfere are those which are reduced at the H.M.D.E. at −0·6 V *versus* S.C.E. or before this value is reached, resulting in peaks that merge with the peak for lead or contribute significantly to the background current. The most important of these elements that occur in steel samples are molybdenum, tin and copper, and the interference caused by each of them was examined in detail.

Interference due to copper—The electro-reduction of Cu^{2+} ions at the H.M.D.E. occurs at about +0·05 V *versus* S.C.E., indicating that at −0·6 V *versus* S.C.E. any copper present in the sample solution will contribute to the background current. When the same medium was used for both electrolysis and stripping, copper at a concentration of only 3×10^{-5} M (0·19 per cent. of copper in a solution containing 1·0 g l^{-1} of steel) could be tolerated (Fig. 7).

However, as previously mentioned, the technique of medium exchange has been used before to overcome such problems and Fig. 8 shows the effect of increasing copper concentration on the stripping voltammogram of a 1×10^{-8} M solution of lead (0·0002 per cent. of lead), using a de-oxygenated 1×10^{-3} M solution of zinc that was 1×10^{-1} M in hydrogen ions and contained 0·5 per cent. of ascorbic acid as the stripping medium. The tolerance limit for copper concentration has now been raised to 1×10^{-4} M (0·63 per cent. of copper).

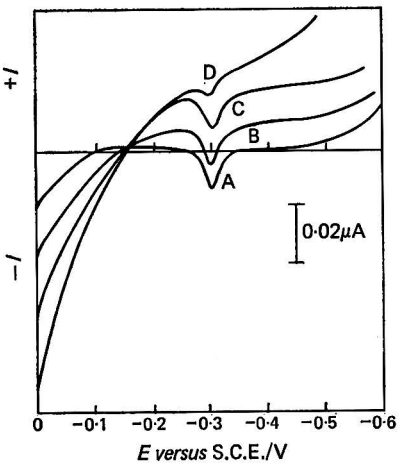

Fig. 7. Interference due to copper (direct). Stripping peaks for a solution containing the equivalent of 0·004 per cent. of lead in a steel in the presence of increasing amounts of copper. The solutions contained 1·0 g l^{-1} of iron, 0·5 per cent. of ascorbic acid and $1·0 \times 10^{-3}$ M of Zn^{2+}. Electrolysis time, 15 minutes, electrolysis potential, −0·6 V *versus* S.C.E. and scanning rate, 0·4 V min^{-1}. All four solutions contained 0·004 per cent. of lead, with B, +0·063; C, +0·189; and D, + 0·252 per cent. of copper

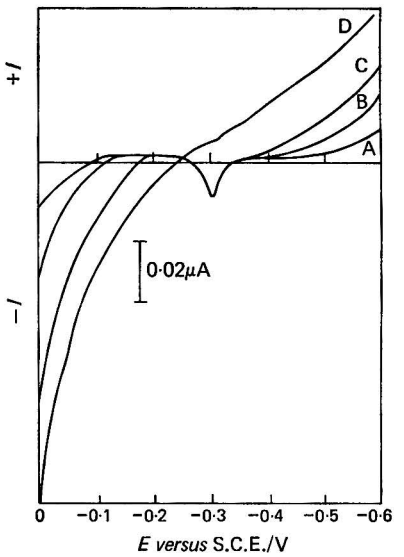

Fig. 8. Interference due to copper (medium exchange into 0·1 M nitric acid). Stripping peaks for a solution containing the equivalent of 0·0004 per cent. of lead in a steel in the presence of increasing amounts of copper. The solutions contained 1·0 g l^{-1} of iron, 0·5 per cent. of ascorbic acid and $1·0 \times 10^{-3}$ M of Zn^{2+}. Electrolysis time, 15 minutes, electrolysis potential, −0·6 V *versus* S.C.E. and scanning rate, 0·4 V min^{-1}. All four solutions contained 0·0004 per cent. of lead, with B, +0·063; C, +0·63; and D, +1·26 per cent. of copper

The sharp rise in anodic current at about 0·0 V *versus* S.C.E. is due to the stripping peak of copper, but the large cathodic current at −0·4 V *versus* S.C.E., which increases with copper concentration, is not so easily explained.

An increase of this magnitude in the background current is often attributed to the electro-reduction of hydrogen ions, but in these solutions with a concentration of these ions of only 0·1 M this reaction does not usually occur until about −1·0 V *versus* S.C.E. is reached. As a test of this hypothesis, stripping voltammograms were obtained for solutions that were 1×10^{-8} M in lead (0·0002 per cent. of lead) with a single concentration of copper ($3·5 \times 10^{-4}$M; 2·2 per cent. of copper), using a stripping medium of different pH in each instance. The results of this test are shown in Fig. 9, and it can be clearly seen that the cathodic background current is reduced as the pH of the stripping medium is increased.

This large increase in the cathodic background current can therefore be ascribed to the reaction—

$$H^+ + e^- \rightarrow \tfrac{1}{2}H_2 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

The overpotential for this reaction is about −0·8 V at a mercury electrode, but is considerably less, about −0·4 V, at a copper electrode. In solutions containing large concentrations of copper that are electrolysed at −0·6 V *versus* S.C.E., the copper concentration inside the mercury drop will be high because of the reaction—

$$Cu^{2+} + 2e^- \rightarrow (Cu^0Hg) \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Therefore, it is likely that the H.M.D.E. now behaves as a copper amalgam electrode and reaction (2) is catalysed by the presence of copper.

In order to determine any loss in peak current for lead as a result of the presence of copper, stripping peaks were measured for a solution that was 1×10^{-7} M in lead and contained copper at various concentrations, using an acetate buffer medium for the stripping stage. The extent of copper interference on the stripping peak for lead, which is shown in Fig. 10, becomes significant at concentrations of copper above $1{\cdot}5 \times 10^{-4}$ M (approximately 0·95 per cent. of copper in a solution containing 1·0 g l^{-1} of steel). This level is a considerable improvement on the previous tolerance limits for copper, but solutions containing copper above this concentration can be analysed if a new calibration graph is prepared with copper present. However, it can be concluded that steel samples that contain up to 0·95 per cent. of copper, which is sufficiently high for most types of steels, can be analysed successfully.

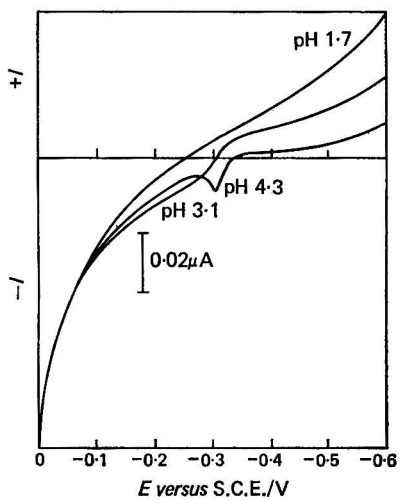

Fig. 9. Interference due to copper (medium exchange pH variation). Stripping peaks for a solution containing the equivalent of 0·0004 per cent. of lead and 2·2 per cent. of copper in a steel. The stripping was carried out in solutions at various pH values. Sample solutions and electrolysis conditions as described in Fig. 7

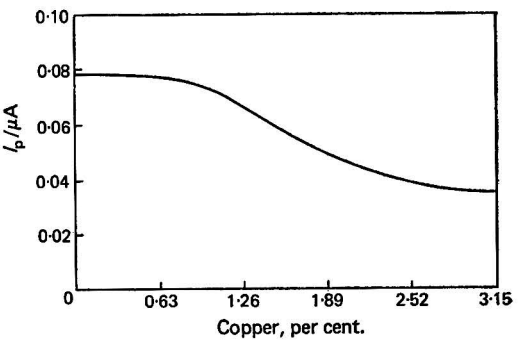

Fig. 10. Interference due to copper (medium exchange). The effect of copper content on the stripping peak of a steel sample containing 0·002 per cent. of lead. The stripping was carried out in a sodium acetate solution (pH 4·3) by using procedure C. Sample solutions and electrolysis conditions as described in Fig. 7)

Interference due to molybdenum—Polarographic waves due to the reduction and oxidation of various ionic states of molybdenum also occur before −0·6 V *versus* S.C.E. is reached and therefore contribute to the background current. Stripping peaks should not be obtained, however, as molybdenum cannot be plated into a mercury cathode and in acidic media gives soluble reduced species.[14] However, according to Lagrange and Schwing,[14] molybdenum(VI) at pH 5 is reduced at a mercury electrode to a solid product, $MoO_2.2H_2O$, which can then be determined by anodic stripping.

When molybdenum was added to solutions containing iron, zinc and ascorbic acid, and anodic stripping analysis carried out for lead, interference occurred at 2×10^{-6} M molybdenum concentration (0·02 per cent. of molybdenum), which consisted of both polarographic waves and stripping peaks (Fig. 11). When medium exchange was used, the limit of interference was raised to 1×10^{-5} M concentration (Fig. 12), which is equivalent to approximately 0·1 per cent. of molybdenum in solutions containing 1·0 g l^{-1} of steel, and this concentration of molybdenum is therefore the maximum that can be tolerated in a steel sample; it should be sufficient in most instances except for stainless steels, which often contain more molybdenum.

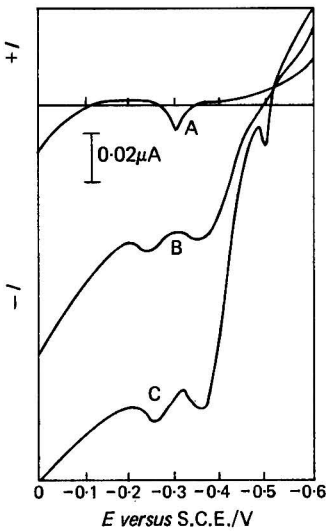

Fig. 11. Interference due to molybdenum (direct). Stripping peaks for a solution containing the equivalent of 0·0004 per cent. of lead in a steel in the presence of increasing amounts of molybdenum. Sample solutions and electrolysis conditions as described in Fig. 7. All three solutions contained 0·0004 per cent. of lead, with B, +0·02; and C, +0·05 per cent. of molybdenum

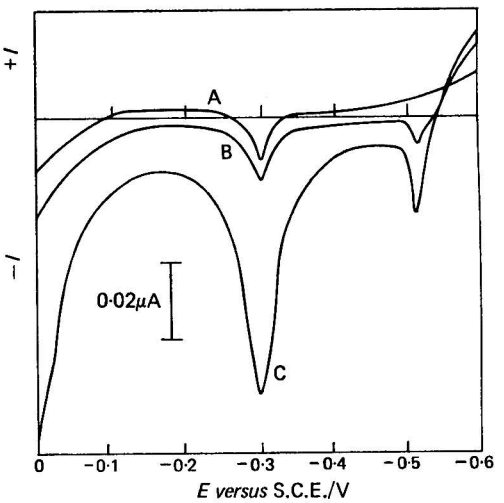

Fig. 12. Interference due to molybdenum (medium exchange). Stripping peaks for a solution containing the equivalent of 0·0004 per cent. of lead in a steel in the presence of increasing amounts of molybdenum. The stripping was carried out in a sodium acetate solution (pH 4·3) by using procedure C. Sample solutions and electrolysis conditions as described in Fig. 7. All three solutions contained 0·0004 per cent. of lead, with B, +0·1; and C, +0·2 per cent. of molybdenum

Interference due to tin—Tin is reduced at a mercury electrode in acidic media at about −0·45 V *versus* S.C.E. and has been determined in steel samples by Phillips and Shain[11] who used anodic stripping voltammetry. The presence of tin in sample solutions, by increasing the background current and giving a stripping peak that merges with the peak for lead, will therefore interfere. This interference was found to occur when tin at the low concentration of 5×10^{-7} M was added to test solutions just prior to electrolysis, a large peak resulting at −0·42 V *versus* S.C.E. even when medium exchange was employed.

However, when the same amount of tin was added to the steel sample at the dissolution stage and the usual analysis carried out, no such peak occurred. Additions of tin in the same manner at concentrations up to 1×10^{-5} M (equivalent to 0·1 per cent. of tin on a 1·0 g l^{-1} steel sample) caused no interference. The most likely explanation of this behaviour is that the added tin is precipitated in the nitric acid medium at the dissolution stage, together with any silicon and carbon present in the steel sample, which is then filtered off. Thus, it can be concluded that tin does not interfere.

ANALYSIS OF STEEL SAMPLES—

A wide variety of steel and cast-iron samples were analysed by using procedure A and the results are shown in Table I. The electrolysis time and mode of stripping (procedure

TABLE I

ANALYSIS OF STANDARD STEEL SAMPLES

Sample	Lead, per cent. Certificate value	Found	Procedure	T_d/ minutes
BCS 330	0·003	0·0022	Direct	15
BCS 328	0·015	0·014		3
BCS 334	0·0011	0·0010		15
BCS 335	0·0015	0·0013		15
Cast iron 1	0·0028*	0·0022		15
2	0·014*	0·013		3
3	0·0022*	0·0024		15
4	0·0038*	0·0038		15
5	0·0018*	0·0016		15
6	0·007*	0·0073		3
BCS 328	0·015	0·015	Medium exchange	3
BCS 326	0·014	0·013		3
Cast iron (A)	0·0018*	0·0019		15
(B)	0·0004*	0·0004		15
Cast iron (A) + 0·1 per cent. of tin	0·0018	0·0017		15
Cast iron (B) + 0·1 per cent. of tin	0·0004	0·0004		15

* BCIRA values.

B or C) chosen depended on the sample composition. As can be seen from Table I the results compare satisfactorily with the certificate values or, for the cast-irons, with the results obtained in the BCIRA laboratory. The time taken for a single analysis depends on the lead content of the sample, but even at the lowest levels of lead, it is possible to complete the analysis together with a standard addition, if required, in 1 hour, a considerable saving in time over the methods in current use, which can take several hours to complete.

A further advantage is that the method is direct, no solvent extractions or chemical operations being necessary other than sample dissolution. The risk of contamination and losses by adsorption or in the solvent extraction step is therefore virtually eliminated, resulting in greater accuracy. The precision of the method is demonstrated in Table II and the measured coefficient of variation (8 per cent.) should be acceptable for such a low level of lead. The detection limit of the method (Table III) was calculated to be 1 p.p.m. (1×10^{-4} per cent. of lead) in the steel sample.

TABLE II

REPRODUCIBILITY TEST

Lead, per cent.	
BCS 328 (0·015 per cent. of lead)	Cast iron (A) (0·0018 per cent. of lead)
0·013	0·0019
0·0125	0·0020
0·012	0·0019
0·0125	0·0017
0·0145	0·0019
0·014	—
0·0115	—
0·013	—
0·0135	—
0·015	—
0·014	—
0·0115	—
0·0135	—
0·0135	—
0·0125	—
Mean .. .. .. 0·013	0·0019
Coefficient of variation 8 per cent.	6 per cent.
Standard deviation .. 0·001	0·0001

Interferences to the method are few, only those of copper and molybdenum being of any significance. The maximum allowable level of molybdenum in the steel sample was found to be 0·1 per cent. With copper, up to about 0·2 per cent. in the steel can be tolerated, but this level is raised to 0·9 per cent. when medium exchange is used. The medium exchange step is not difficult to apply, but some care is necessary in ensuring that the solution transfer is carried out as quickly as possible. Anodic stripping voltammetry has been applied previously to the determination of copper[12] and tin[11] in steels and there is no reason why steel could not also be analysed for other amalgam-forming elements, *e.g.*, bismuth, antimony, etc. If these elements were present in the sample at suitable concentrations it may even be possible to determine them simultaneously.

TABLE III

DETECTION LIMIT FOR THE DETERMINATION OF LEAD IN STEEL

Test solutions contained 1·0 g l^{-1} of iron and 0·5 per cent. of ascorbic acid, and were 0·1 M in H^+ and 1×10^{-3} M in Zn^{2+} ions

Electrolysis potential, −0·6 V *versus* S.C.E.; electrolysis time, 15 minutes; and rate of voltage scan, 0·4 V min^{-1}

Lead added, per cent.	Peak current/μA
0·0	0·0025, 0·0035, 0·0030
$0·5 \times 10^{-4}$	0·0050, 0·0040, 0·0060
$1·0 \times 10^{-4}$	0·0050, 0·0060, 0·0060
$1·5 \times 10^{-4}$	0·0070, 0·0085, 0·0060
$2·0 \times 10^{-4}$	0·0090, 0·0110, 0·0100

Variation about the calibration graph corresponds to a standard deviation of $0·29 \times 10^{-4}$ per cent. of lead.
∴ Detection limit = 4S = $1·16 \times 10^{-4}$ per cent. of lead (1·16 p.p.m.)

One of us (B.M.) thanks the Shotton Works of the British Steel Corporation for financial support during this work, as well as Mr. A. E. Burgess, Glasgow College of Technology, for some useful discussion.

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Received *November 27th*, 1973
Accepted *February 5th*, 1974

Analyst, August, 1974, Vol. 99, *pp.* 469–470

A Modification to the Extraction - Atomic-absorption Method for the Determination of Antimony, Bismuth, Lead and Tin

BY K. THORNTON

(Henry Wiggin and Co. Ltd., Holmer Road, Hereford)

AND KEITH E. BURKE

(The International Nickel Company, Inc., Paul D. Merica Research Laboratory, Sterling Forest, Suffern, New York 10901, *U.S.A.)*

It has been shown that the use of organometallic compounds for the calibration procedure used in the extraction - atomic-absorption determination of antimony, bismuth, lead and tin in metallurgical materials leads to erroneous results at low concentration levels. Results are presented, which demonstrate that the technique is satisfactory when calibrated with solutions that have been taken through the extraction procedure.

THE previously published analytical method for the determination of trace amounts of antimony, bismuth, lead and tin[1] depends on the extraction of the iodides of these elements into a solution of tri-n-octylphosphine oxide (TOPO) in 4-methylpentan-2-one. The extract is then nebulised directly into the atomic-absorption flame. The calibration procedure involves the use of solutions of organometallic compounds in 4-methylpentan-2-one. It is shown in Table V of the original publication[1] that agreement between this procedure and two other atomic-absorption techniques is generally acceptable at higher concentration levels of the elements determined. Furthermore, the accuracy is shown to be satisfactory at these higher levels for the analysis of standardised samples (Table VI of the original publication).

At lower concentration levels, particularly below 10 p.p.m., the agreement between the three techniques is poor and it is difficult to assess the accuracy of the extraction procedure as no certified standards are available at these levels.

Subsequent work has shown that the calibration procedure that makes use of organometallic compounds dissolved in 4-methylpentan-2-one gives rise to erroneous results, the values being, in general, higher than the true values. However, a simple modification, in which aqueous solutions of the elements determined are carried through the extraction procedure and treated in the same way as the sample solutions, overcomes this difficulty. This modification avoids any error that might be introduced by variation in the extraction conditions or errors due to light scattering, molecular absorption or organometallic bonding.[2]

The revised procedure has been applied to several of the samples included in Table VI of the original publication. Originally, two alternative atomic-absorption techniques were used for comparison purposes. The comparison procedure with a simple 4-methylpentan-2-one extraction is subject to the same problems that have been encountered with the proposed method, with TOPO in solution in 4-methylpentan-2-one, whereas the separation technique based on co-precipitation with manganese dioxide is inherently less sensitive and consequently inaccurate at levels below about 10 p.p.m. In an effort to overcome these difficulties alternative analytical procedures have been developed that involve solvent-extraction separations followed by square-wave polarographic determination. Lead was extracted as the iodide complex in a method similar to that proposed by Luke,[3] and bismuth, tin and antimony were extracted as their complexes with isooctylthioglycollate.[4]

The results obtained by using the modified extraction - atomic-absorption procedure and the polarographic methods are given in Table I.

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TABLE I

DETERMINATION OF ANTIMONY, BISMUTH, LEAD AND TIN IN COMMERCIALLY AVAILABLE MATERIALS

		Found, p.p.m.							
		Antimony		Bismuth		Lead		Tin	
Designation	Matrix	A*	B*	A	B	A	B	A	B
HW E-3923	†INCOLOY alloy 800	6	4	<0·5	<0·5	1·0	1·7	17	25
HW E-3924	INCOLOY alloy 800	13	13	<0·5	<0·5	1·2	1·2	20	24
HW E-3925	INCOLOY alloy 800	11	12	<0·5	<0·5	1·0	1·8	23	24
HW E-3926	INCOLOY alloy 800	12	12	<0·5	<0·5	0·8	1·4	24	23
HW E-3927	INCOLOY alloy 800	13	13	<0·5	<0·5	1·0	1·4	20	21
HW E-3928	INCOLOY alloy DS	12	12	<0·5	<0·5	0·9	1·4	20	22
HW E-3929	INCOLOY alloy DS	9	11	<0·5	<0·5	1·0	0·9	19	17
HW E-3930	INCOLOY alloy DS	7	9	<0·5	<0·5	1·0	0·9	19	20
HW E-3931	INCOLOY alloy DS	9	10	<0·5	<0·5	1·0	1·1	13	19
HW E-3932	INCOLOY alloy DS	9	11	<0·5	<0·5	1·0	1·1	19	19
HW B-7047	†INCONEL alloy X750	1	1	<0·5	<0·5	1·5	1·5	7	6
HW B-7048	INCONEL alloy X750	<1	1	<0·5	<0·5	1·2	1·3	7	6
HW B-7049	INCONEL alloy X750	<1	1	<0·5	<0·5	1·2	1·2	3	6
HW B-7050	INCONEL alloy X750	<1	1	<0·5	<0·5	1·3	1·6	3	4
HW B-7051	INCONEL alloy X750	<1	1	<0·5	<0·5	1·3	1·3	3	4
BCS 310	†NIMONIC alloy 90	4	5	<0·5	<0·5	5·7	5·5	21	24
BCS 310/1	NIMONIC alloy 90	4	5	<0·5	<0·5	15	15	35	34
BCS 387	NIMONIC alloy 901	3	1	<0·5	<0·5	0·5	0·7	19	18
BCS 371	Commercial nickel	1	—	<0·5	—	14	—	<1	—
NBS 671	Nickel oxide	<1	<1	<0·5	<0·5	14	16	<2	3
NBS 672	Nickel oxide	<1	—	<0·5	<0·5	35	39	<2	4
HW F-292	Nickel	2	<1	<0·5	<0·5	0·8	0·8	<1	3
HW F-293	Nickel	<1	<1	<0·5	<0·5	0·8	0·8	<1	<1
HW F-294	Nickel	<1	<1	<0·5	<0·5	1·4	1·7	<1	<1
HW F-295	Nickel	<1	<1	<0·5	<0·5	0·9	0·5	<1	<1
HW F-296	Nickel	<1	<1	<0·5	<0·5	1·1	1·0	<1	<1
HW F-297	Nickel	<1	<1	<0·5	<0·5	0·3	0·5	<1	<1

* Laboratory A used the modified TOPO - atomic-absorption procedure, while laboratory B used solvent extraction - polarographic methods.

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Received *July 2nd*, 1973
Accepted *February 20th*, 1974

Spectrophotometric Determination of Magnesium in Tobacco Leaves with Eriochrome Black B

By G. SLEGERS and A. CLAEYS

(*Laboratory of Analytical Chemistry, Faculty of Pharmaceutical Sciences, State University of Ghent, 9000 Ghent, Belgium*)

A spectrophotometric method for the determination of magnesium in tobacco leaves with Eriochrome black B is described. After destruction of the tobacco leaves with a mixture of nitric, perchloric and sulphuric acids, the magnesium is separated from interfering ions by ion-exchange chromatography on Dowex 50W-X12, 100 to 200 mesh, and determined spectrophotometrically after coupling it with Eriochrome black B. The accuracy of the method was tested against a reference material.

Various methods, such as atomic-absorption spectrophotometry[1] and spark-source mass spectrometry,[2] are applied to the determination of magnesium in tobacco products. In spectrophotometry, several lake-forming sensitive reagents such as 4-(4-nitrophenylazo)-1-naphthol[3] and thiazol yellow dyes[4] have been described for the direct determination of magnesium. It is difficult, however, to obtain reproducible results with these dyes, because of variations in experimental conditions.

Eriochrome black T [1-(1-hydroxy-2-naphthylazo)-2-hydroxy-5-nitro-4-naphthalenesulphonic acid], used by Harvey, Komarmy and Wyatt,[5] Gasser[6] and Young and Sweet,[7] is a more suitable reagent. This reagent is sensitive but not as specific as those mentioned above and interferences from calcium, copper, manganese, iron, aluminium, cobalt and nickel have been reported by Diehl, Goetz and Hach.[8] Eriochrome black B [1-(1-hydroxy-2-naphthylazo)-2-hydroxy-4-naphthalenesulphonic acid] does not have any disadvantages compared with Eriochrome black T and was used in the method proposed in this paper for the determination of magnesium because of its greater sensitivity.

Experimental

Apparatus—

All absorbance measurements were carried out with a Beckman, Acta V, spectrophotometer equipped with 1-cm glass cells.

Reagents and materials—

Standard stock magnesium solution, 46·6 *mg* l^{-1}—This solution was prepared with analytical-reagent grade magnesium chloride ($MgCl_2.6\,H_2O$) and was standardised by the gravimetric pyrophosphate procedure. A 1 + 9 dilution of the stock solution was used in the experiments.

Buffer solutions—Buffer solutions (0·1 M) in the pH range 8·5 to 10 were prepared with ammonia solution and ammonium chloride, and a 0·1 M buffer solution with a pH of 10·5 was prepared with piperidine and hydrochloric acid.

Dye solution, 0·5 *per cent.* m/V—Eriochrome black B (0·5 g) was dissolved in methanol and the solution diluted to 100 ml with methanol. Dye solutions were freshly prepared each day and stored in the dark.

Effect of pH study—

Absorption spectra of the dye - magnesium complex were determined in the pH range 8·5 to 10·5 on solutions prepared by mixing 10 ml of 1 + 9 magnesium stock solution with 10 ml of buffer and 2 ml of dye solution in a 100-ml calibrated flask and diluting the mixture to the mark with water. Blanks were prepared in a similar way, omitting the magnesium solution. The absorption spectra, which were measured immediately, showed that the highest absorbance was obtained at λ_{max} 559 nm with the solution at pH 10·5.

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INFLUENCE OF DYE CONCENTRATION—

The colour intensity also depends on the amount of dye present in the solution. This aspect was investigated by adding increasing amounts of 0·5 per cent. *m*/*V* dye solution to a mixture of 10 ml of 1 + 9 magnesium stock solution and 10 ml of buffer of pH 10·5 and diluting the mixture to 100 ml. Blanks were prepared in a similar way, with the magnesium solution omitted. The colour intensity remained constant with concentrations of dye above a fifteen-fold excess (Fig. 1).

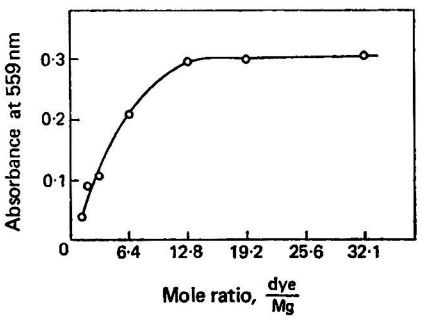

Fig. 1. Influence of dye concentration on the absorption

SEQUENCE OF ADDITION OF REAGENTS—

As it is possible that magnesium may form different complexes with Eriochrome black T, the order of addition of the reagents is important. Four sequences were therefore investigated for Eriochrome black B at pH 10·5, with mixing of the solution after the addition of each component.

For the first sequence 10 ml of 1 + 9 magnesium stock solution, 10 ml of buffer solution and 2 ml of 0·5 per cent. *m*/*V* dye solution in methanol were transferred into a 100-ml calibrated flask and the mixture was diluted to the mark with water; for the second, the order was buffer solution, dye solution, then 1 + 9 magnesium stock solution followed by dilution to 100 ml; for the third, 1 + 9 magnesium stock solution, dye solution, then buffer solution and dilution to 100 ml; and for the fourth, 1 + 9 magnesium stock solution, buffer solution, followed by dilution to nearly 80 ml, then dye solution and dilution to 100 ml. In each instance a blank was prepared in a similar way.

Absorbances of samples and blanks were measured at intervals at 559 nm. From the results (Fig. 2), it can be seen that the third sequence gave the highest absorbance and stability and was used in the following experiments.

COMPOSITION OF THE COMPLEX—

Only one complex is formed between magnesium and Eriochrome black B at pH 10·5, and it was investigated by measuring the absorbance of a series of solutions in which the mole ratio of magnesium to dye reagent was varied between 1:10 and 10:1. For the continuous variation experiments (Fig. 3), the concentrations of both the magnesium and dye solutions were $1{\cdot}8 \times 10^{-3}$ M. Appropriate volumes of the two solutions, totalling 10 ml, were transferred by pipette into 100-ml calibrated flasks, 10 ml of buffer of pH 10·5 were added and the mixtures diluted to 100 ml. The solutions were then stored in the dark for 1 hour. Blanks containing 1 to 10 ml of dye solution were treated in a similar way. From the absorption curves, the absorbance at $\lambda_{max.}$ was deducted, as shown in Fig. 3. The value of n, the number of ligands bound per cation, can be obtained from the relationship $n = \frac{X_{max.}}{1 - X_{max.}}$ where $X_{max.}$ represents the molar fraction of the dye at the point where the difference curve is at a maximum. With $X_{max.}$ equal to 0·66, the value of n is 1·94 or approximately 2 mol of ligand per mole of magnesium.

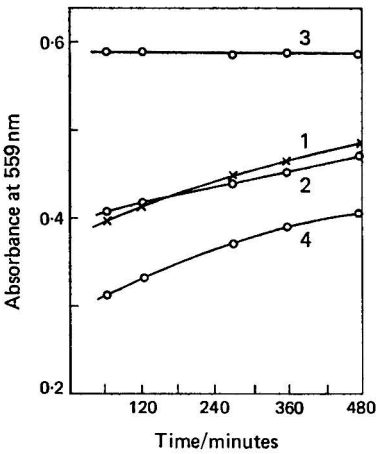

Fig. 2. Development and stability of the colour. Sequence of addition of reagents: 1, first sequence; 2, second sequence; 3, third sequence; and 4, fourth sequence

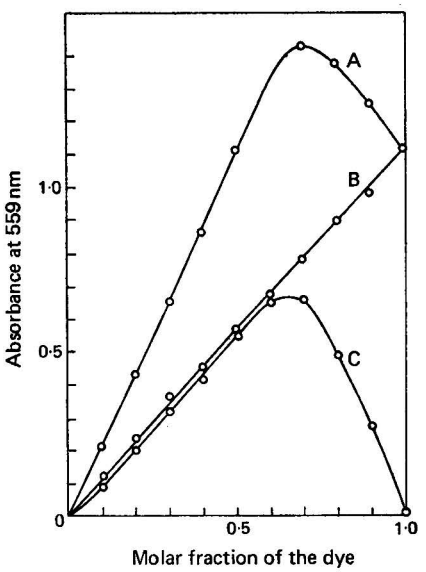

Fig. 3. Composition of the complex. A, Total curve obtained with aliquots of $1{\cdot}8 \times 10^{-3}$ M magnesium and dye solutions totalling 10 ml *plus* 10 ml of buffer solution (pH 10·5) made up to 100 ml and solution stored for 1 hour in the dark (absorbance measured at 559 nm against water); B, curve for 10 ml of $1{\cdot}8 \times 10^{-3}$ M dye solution *plus* 10 ml of buffer solution made up to 100 ml and treated as for A; and C, curve obtained by subtracting B from A, giving absorbance of the magnesium - dye complex

STUDY OF CALIBRATION GRAPH—

The analysis was carried out by use of a calibration graph. Because several factors, such as pH and sequence of addition of reagents, influence the absorbance, values for the calibration graph must be measured under constant experimental conditions. From previous experiments it was decided to construct the curve by using the third sequence of additions, *i.e.*, 10 ml of 1 + 9 magnesium stock solution followed by addition of 2 ml of 0·5 per cent. *m/V* dye reagent in methanol, 10 ml of buffer of pH 10·5, and final dilution to 100 ml.

The effect of time of storage of the reaction solution in the dark was investigated by measuring the absorbance at various intervals. Because Beer's law is followed in the concentration range studied (Table I), the relationship $y = ax + b$ can be applied to the calibration graph.

The factors a and b are calculated by the method of regression analysis (Table I) and the variation about the fitted curve is calculated from the equation—

$$V_Y = \frac{\Sigma(Z - Y)^2}{\Phi}$$

where Φ is the degree of freedom (number of points on the graph − 2); Z the measured absorbance; and Y the absorbance calculated from the regression curve. From Fig. 4 it is deduced that the variation is at a minimum after 4 hours' storage in the dark.

PROCEDURE FOR THE SEPARATION OF MAGNESIUM—

After wet destruction of 1 g of lyophilised tobacco leaves with a mixture of strong oxidising acids consisting of 10 ml of 70 per cent. *m/V* perchloric acid, 10 ml of 35 N sulphuric acid and 10 ml of 14 N nitric acid, the residual acid mixture was transferred quantitatively into a

250-ml calibrated flask and diluted to the mark with water; 3 ml of this solution were neutralised with ammonia solution and poured into a column, 1 cm in diameter and 14 cm in length, loaded with cation exchanger Dowex 50W-X12, 100 to 200 mesh.

After washing the column with water until the eluate was free from acid, it was eluted with 0·7 M hydrochloric acid in order to remove sodium, the first 28 ml of eluate being rejected and the sodium collected in the next 100 ml. The column was then eluted with 0·7 M nitric acid, potassium being collected in the next 190 ml of eluate and magnesium in the following 275 ml. Finally, calcium was eluted with 2 M nitric acid and was collected in 150 ml of eluate. Preliminary experiments had shown that the recovery of these elements was quantitative under these conditions.

TABLE I

CALIBRATION GRAPH WITH MEASUREMENTS AT DIFFERENT STORAGE TIMES

Magnesium taken/g l^{-1}	Absorbance at 559 nm after				
	0 minutes	60 minutes	255 minutes	480 minutes	1440 minutes
$1{\cdot}36 \times 10^{-4}$	0·198	0·128	0·097	0·085	0·060
$2{\cdot}27 \times 10^{-4}$	0·150	0·158	0·167	0·194	0·246
$3{\cdot}64 \times 10^{-4}$	0·390	0·324	0·310	0·308	0·300
$4{\cdot}55 \times 10^{-4}$	0·357	0·365	0·375	0·403	0·468
$5{\cdot}91 \times 10^{-4}$	0·568	0·500	0·498	0·498	0·568
$6{\cdot}82 \times 10^{-4}$	0·512	0·519	0·552	0·588	0·708
$9{\cdot}10 \times 10^{-4}$	0·730	0·738	0·775	0·805	0·935
$1{\cdot}14 \times 10^{-3}$	0·885	0·893	0·950	1·000	1·176
$1{\cdot}36 \times 10^{-3}$	1·073	1·081	1·126	1·160	1·382
a	0·033	0·035	0·038	0·040	0·048
b	0·062	0·013	−0·010	−0·012	−0·043
V_Y	$3{\cdot}0 \times 10^{-3}$	$4{\cdot}9 \times 10^{-4}$	$1{\cdot}8 \times 10^{-4}$	$3{\cdot}0 \times 10^{-4}$	$1{\cdot}2 \times 10^{-3}$

The eluate fraction containing magnesium was evaporated to dryness, the residue dissolved in 5 ml of 0·1 M hydrochloric acid and the solution transferred quantitatively into a 100-ml calibrated flask and diluted to the mark with water. A 10-ml aliquot of this solution was then mixed with 2 ml of 0·5 per cent. *m/V* dye solution and 10 ml of buffer solution of pH 10·5 and the mixture diluted to 100 ml. After storing the solution and a blank for 4 hours in the dark, their absorbances were measured at 559 nm.

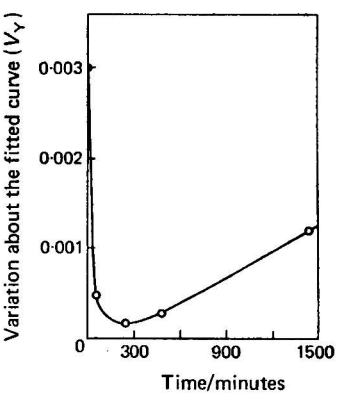

Fig. 4. Slope of the variation about the fitted curve

RESULTS AND DISCUSSION

Various samples of tobacco leaves were subjected to the described procedure and the magnesium contents were read off from the calibration graph and corrected for dilution. The results are given in Table II.

In order to check the accuracy of the method, the magnesium content of orchard leaves, a standard reference material SRM 1571, supplied by the National Bureau of Standards, was determined by the described method. The result, shown in Table II, agreed with the value given by the National Bureau of Standards.

TABLE II

DETERMINATION OF MAGNESIUM IN TOBACCO LEAVES IMPORTED INTO THE BELGIAN - LUXEMBURG ECONOMIC UNION

Brand	Magnesium content, per cent. *m/m*	Brand	Magnesium content, per cent. *m/m*
U.S.A., burley	0·41	India, flue cured	0·45
U.S.A., fire cured	0·43	Turkey, sun cured	0·43
U.S.A., air cured	0·48	Malawi, burley, air cured	0·46
U.S.A., flue cured	0·38	Malawi, fire cured	0·46
Indonesia, air cured	0·45	Belgium, air cured	0·41
Greece, sun cured	0·46	Orchard leaves SRM 1571	0·65
Rhodesia, flue cured	0·46	Mean error, per cent.	2·0
		NBS value	0·62 ± 0·02

CONCLUSION

A spectrophotometric method has been established for the determination of magnesium in tobacco leaves with an error of ±2 per cent. The calibration graph has been studied in the concentration range $1{\cdot}4 \times 10^{-4}$ to $1{\cdot}3 \times 10^{-3}$ g l^{-1} of magnesium. However, the method is sensitive enough to permit determinations to be made in the microgram range. The method has been applied to the determination of magnesium in tobacco leaves imported into the Belgian - Luxemburg Economic Union.

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Received *January 29th*, 1974
Accepted *March 7th*, 1974

The Spectrophotometric Determination of Ampicillin and Cloxacillin in Combined Injections*

By A. G. DAVIDSON and J. B. STENLAKE

(*Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow, G1 1XW*)

A method is described for the determination of ampicillin and cloxacillin in injections. Ampicillin is determined by an absorbance difference technique based on the higher absorbance of ampicillin at 268 nm in a solution at pH 5 than in one at pH 9. Cloxacillin is determined by measurement of the absorbance at 275 nm and the application of a small correction for the absorbance of ampicillin. The accuracy, precision and specificity of the method are discussed. The analytical results obtained for commercial samples of ampicillin - cloxacillin (1 + 1 and 2 + 1) mixtures are compared with those obtained by microbiological assay.

The methods that are currently used to determine penicillins in pharmaceutical preparations have been reviewed.[1] They include measurement of the absorbance at 320 to 360 nm of the copper-stabilised penicillenic acid, formed by isomerisation of the penicillin in acidic solution, iodimetric assay after alkaline cleavage of the β-lactam ring and microbiological assay. The non-specificity of these methods, however, renders them unsuitable for the analysis of mixtures of different penicillins, and separation of the individual penicillins is usually necessary prior to their determination.

One mixture of penicillins available commercially contains ampicillin sodium and cloxacillin sodium in 1:1 and 2:1 proportions, as the free acids. These preparations are supplied in vials as a dry powder for injection after reconstitution with water. The component penicillins have been determined microbiologically following separation on a column of basic acetate resin[2] and after separation by a gel-electrophoretic method based on that of Lightbown and de Rossi[3] (D. Sykes, personal communication). A chemical determination[4] of ampicillin and cloxacillin has been described in which ampicillin is determined colorimetrically after reaction with ninhydrin, and cloxacillin is determined by measurement of the absorbance at 345 nm, 12 minutes after dissolving the sample in hydrochloric acid solution.

In the present work we have determined both penicillins by the spectrophotometric analysis of solutions prepared from a single sample weighing. Ampicillin is determined by difference spectrophotometry because at 250 to 275 nm the absorbance of ampicillin is greater in solution at pH 5 than at pH 9, while the absorbance of cloxacillin is unchanged by variation in pH. Thus, the absorbance at 268 nm of a solution of the penicillin mixture in pH 5 buffer, read against a solution of the mixture of equal concentration in pH 9 buffer, is proportional to the concentration of ampicillin. Cloxacillin is determined by measurement of the absorbance of the sample at 275 nm and correction for the absorbance of ampicillin.

Experimental

Spectrophotometer—

Absorbance values were measured in 1-cm cells that were matched for equal path length on a Unicam SP1800 spectrophotometer working in the double-beam mode. The most sensitive absorbance range, 0 to 0·2, was used for measurement of the absorbance differences of ampicillin, and the range 0 to 1·0 was used for the measurement of cloxacillin absorbance. The slit-width, 0·5 mm, remained constant throughout the assay.

Reagents—

Ampicillin sodium and cloxacillin sodium reference compounds—These were kindly supplied by Beecham Pharmaceuticals, Worthing, Sussex. The British Pharmacopoeial assay results, calculated as the free acids, supplied by the manufacturer were 91·3 per cent. ($C_{16}H_{19}N_3O_4S$) and 89·8 per cent. ($C_{19}H_{18}ClN_3O_5S$), respectively.

* The preliminary results of this work were presented at the British Pharmaceutical Conference, September 1973, London.

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"Double-strength" pH 9 buffer—Dissolve potassium chloride (7·46 g), boric acid (6·18 g) and 1 N sodium hydroxide solution (41·6 ml) in water and dilute the solution to 1 litre.

"Double-strength" pH 5 buffer—Dissolve sodium acetate trihydrate (38·10 g) and glacial acetic acid (7·2 g) in water and dilute the solution to 1 litre.

"Single-strength" pH 5 buffer—Dilute double-strength pH 5 buffer with an equal volume of water.

Procedure

Ampicillin—

Dissolve the ampicillin - cloxacillin mixture (about 65 mg of the 1 + 1 mixture or about 48 mg of the 2 + 1 mixture, accurately weighed) in water and make the volume up to 25 ml. By use of a pipette transfer 10-ml volumes into two flasks, one containing double-strength pH 5 buffer (10 ml) and the other double-strength pH 9 buffer (10 ml). Immediately measure the absorbance of the pH 5 buffered solution with the pH 9 solution in the reference cell at the difference maximum, which is at a wavelength of about 268 nm.

Measure the absorbance difference of a similarly prepared solution of ampicillin sodium (about 32 mg, accurately weighed).

Cloxacillin—

Measure the absorbance of the pH 5 buffered solution of the sample (prepared above) at 275 nm with single-strength pH 5 buffer in the reference cell and, similarly, measure the absorbance of the standard solution of ampicillin sodium in pH 5 buffer (also prepared above). Then measure the absorbance of a standard solution of cloxacillin sodium (about 32 mg in 50 ml for the 1 + 1 mixture or 32 mg in 100 ml for the 2 + 1 mixture) in single-strength pH 5 buffer.

Calculation—

The concentration (c) of ampicillin ($C_{16}H_{19}N_3O_4S$) in the sample (per cent m/m) is given by

$$c = \frac{\Delta A_{268}(\text{sample})}{\Delta A_{268}(\text{standard})} \times \frac{\text{amount of ampicillin sodium standard (mg)}}{\text{amount of sample (mg)}} \times \text{purity of ampicillin sodium standard (per cent.)}$$

where ΔA_{268} is the absorbance difference at 268 nm, and the percentage purity of the standard is expressed in terms of the free acid.

By using the calculated concentration of ampicillin in the sample and the absorbance at 275 nm of the standard ampicillin solution, calculate the contribution of ampicillin to the absorbance at 275 nm of the sample solution. Then calculate the cloxacillin concentration from the corrected absorbance at 275 nm of the sample solution, using the appropriate ratios and amount of standard in a similar equation.

Results

Effect of pH on the absorbance of ampicillin and cloxacillin—

The ultraviolet absorption spectra of ampicillin and cloxacillin in pH 5 and pH 9 buffers are shown in Fig. 1(*a*) and (*c*). Fig. 1(*b*) shows the absorbance difference spectrum of ampicillin, obtained for the solution buffered at pH 5 with a solution of identical concentration in pH 9 buffer in the reference cell. Peaks occur in the difference spectrum at wavelengths of 256, 262 and 268 nm.

The effect of pH on the absorbance of ampicillin and cloxacillin was further investigated by measuring the absorbance at 268 nm of standard solutions of the penicillins in various buffers in the pH range 3 to 10 (Fig. 2). The absorbance of cloxacillin is constant in this pH range, while ampicillin shows maximum absorbance below pH 5 and minimum absorbance above pH 9. The mid-point of the sigmoid curve for ampicillin occurs at a pH of 7·30, which is in agreement with the pK_2 value of ampicillin of 7·25.[5]

ACCURACY OF THE METHOD—

The accuracy of the method was investigated by analysing several standard mixtures of ampicillin sodium and cloxacillin sodium. The results are given in Table I.

PRECISION—

In order to determine the precision of the method ten replicate analyses were made on each of a 1 + 1 and a 2 + 1 ampicillin - cloxacillin mixture. The relative standard deviations and limits of error for a single determination ($P = 0.95$) for each mixture are recorded in Table II.

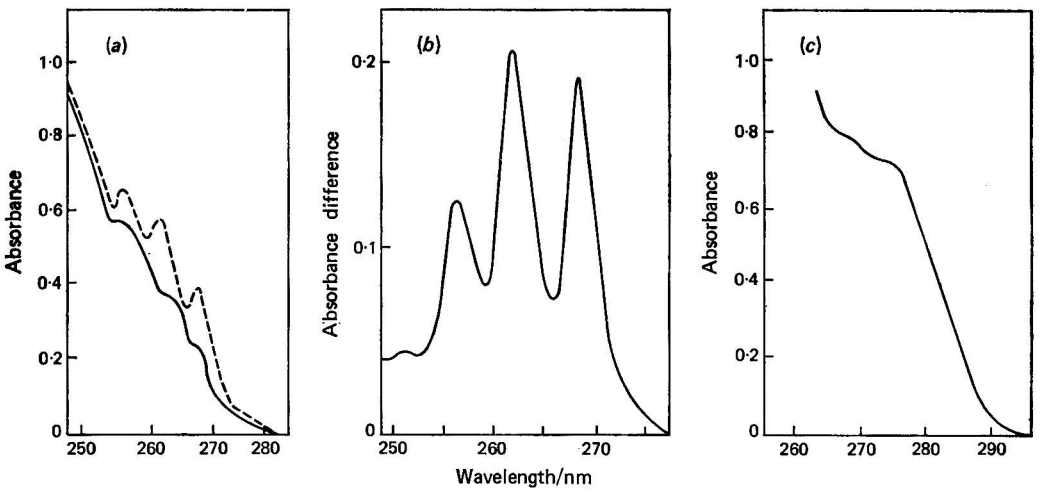

Fig. 1. Ultraviolet spectra of ampicillin and cloxacillin. (*a*) Ultraviolet spectra of ampicillin (0·06 per cent. *m*/*V*) in pH 5 (– – –) and pH 9 (——) buffer. (*b*) Difference spectrum of ampicillin (0·06 per cent. *m*/*V*). (*c*) Ultraviolet spectra of cloxacillin (0·08 per cent. *m*/*V*) in pH 5 and pH 9 buffer. At these pH values the graphs are superimposed

STABILITY OF THE PENICILLINS—

The extent of hydrolysis of the penicillins under the conditions of the assay was investigated. Aliquots of the standard solutions of ampicillin and cloxaxillin at pH 5 and pH 9, alone and combined, were examined for unhydrolysed penicillins, 30 minutes after preparation of the solutions, by the iodimetric method of Finholt, Jurgensen and Kristiansen.[6] The results are given in Table III.

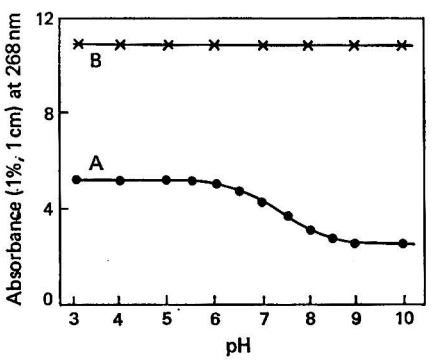

Fig. 2. The effect of pH on the absorbance (1 per cent., 1 cm) of ampicillin (A) and cloxacillin (B) at 268 nm

TABLE I

DETERMINATION OF AMPICILLIN AND CLOXACILLIN, AS THE FREE ACIDS, IN STANDARD MIXTURES OF AMPICILLIN SODIUM AND CLOXACILLIN SODIUM

Ampicillin			Cloxacillin		
Added, per cent. *m/m*	Found, per cent. *m/m*	Recovery, per cent.	Added, per cent. *m/m*	Found, per cent. *m/m*	Recovery, per cent.
45·6	45·2	99·1	44·9	44·9	100·0
44·5	43·9	98·7	46·0	45·4	98·7
45·6	45·2	99·1	44·9	44·4	98·9
46·4	45·8	98·7	44·2	44·3	100·2
35·1	35·8	102·0	55·3	55·1	99·6
55·3	55·3	100·0	35·4	35·8	101·1
61·8	62·1	100·5	28·9	28·6	99·0
59·6	59·2	99·3	31·1	31·5	101·3
60·3	60·7	100·7	30·4	30·2	99·3
61·0	60·8	99·7	29·7	29·3	98·7
50·1	50·2	100·2	40·5	40·5	100·0
41·0	40·4	98·5	49·5	49·6	100·2

ADHERENCE TO BEER'S LAW—

Two series of mixtures were prepared. In one series the amount of cloxacillin sodium was constant at 32 ± 1 mg and that of ampicillin sodium ranged from 0 to 40 mg. In the other series, the amount of ampicillin sodium was constant at 32 ± 1 mg and the cloxacillin sodium ranged from 0 to 40 mg. In the first series ΔA_{268} was proportional to the ampicillin concentration, and in the second series the corrected A_{275} value was proportional to the concentration of cloxacillin, thus confirming that Beer's law is obeyed for both penicillins.

TABLE II

RELATIVE STANDARD DEVIATIONS (RSD) AND LIMITS OF ERROR (LE) OF A SINGLE ASSAY ($P = 0{\cdot}95$) FOR TEN REPLICATE ASSAYS OF 1+1 AND 2+1 AMPICILLIN - CLOXACILLIN MIXTURES

	Ampicillin		Cloxacillin	
	RSD, per cent.	LE, per cent.	RSD, per cent.	LE, per cent.
Ampicillin - cloxacillin (1+1)	0·84	±1·90	1·03	±2·33
Ampicillin - cloxacillin (2+1)	0·80	±1·81	1·62	±3·66

SPECIFICITY—

A number of penicillins were examined for absorbance differences at 268 nm in solutions buffered at pH 5 and 9. The results, expressed as $\Delta\epsilon_{268}$ for the anhydrous penicillin (free acid), are recorded in Table IV.

ASSAY RESULTS—

Commercial batches of ampicillin - cloxacillin (1 + 1 and 2 + 1) injections, supplied by Beecham Pharmaceuticals, were assayed. The results, in Table V, are compared with those

TABLE III

LEVELS OF UNHYDROLYSED PENICILLINS REMAINING 30 MINUTES AFTER PREPARATION OF THE SOLUTIONS

Penicillin	pH	Amount remaining unhydrolysed, per cent.
Ampicillin	5	100·0
Ampicillin	9	99·8
Cloxacillin	5	99·4
Cloxacillin	9	98·9
Ampicillin - cloxacillin (1+1)	5	99·8
Ampicillin - cloxacillin (1+1)	9	99·5

obtained by the manufacturer by using a microbiological method of assay after separation of the penicillins by gel electrophoresis.

TABLE IV

ABSORBANCE DIFFERENCE AT 268 nm OF SEVERAL PENICILLINS UNDER THE CONDITIONS OF THE ASSAY FOR AMPICILLIN

The results are expressed as $\Delta\epsilon_{268}$ for the anhydrous penicillin (free acid)

Penicillin	$\Delta\epsilon_{268}$
Ampicillin sodium	105·6
Ampicillin trihydrate	105·2
Penicillinase-hydrolysed ampicillin	104·7
Amoxycillin	−576·4
Pivampicillin	Precipitated
Cloxacillin	0
Flucloxacillin	0
Benzylpenicillin	0
Phenoxymethylpenicillin	0
Phenethicillin	0
Methicillin	0
Carbenicillin	0
Propicillin	0

DISCUSSION

The higher absorbance of ampicillin in an acidic solution compared with that in an alkaline solution, upon which the determination of ampicillin depends, is caused by protonation of the aminobenzyl side chain substituent in acidic solution. This protonation abolishes the interaction with the π-electron ring resonance, which occurs in alkaline solution, and which results in a blurring of the spectrum [Fig. 1(*a*)]. A similar effect has been observed for cyclizine.[7]

Although the peak in the difference spectrum of ampicillin [Fig. 1(*b*)] at 262 nm is larger than that at 268 nm, the noise associated with the absorbance measurement of the sample at 262 nm is also increased, owing to the higher absorbance of cloxacillin at this wavelength. Measurements of absorbance differences were therefore made at 268 nm.

TABLE V

CONCENTRATION OF AMPICILLIN AND CLOXACILLIN, AS THE FREE ACIDS, IN COMMERCIAL MIXTURES OF AMPICILLIN SODIUM AND CLOXACILLIN SODIUM

Batch	Ampicillin, per cent. *m/m*		Cloxacillin, per cent. *m/m*	
	Spectrophotometric*	Microbiological†	Spectrophotometric*	Microbiological†
A	43·6	43·8	46·2	43·3
B	43·7	45·1	45·9	44·1
C	43·7	45·7	46·2	46·0
D	43·6	44·7	46·6	44·7
E	44·6	44·2	46·9	45·8
F	44·0	43·4	45·8	43·5
G	59·9	57·7	29·8	29·1
H	58·7	57·0	30·8	30·5
I	59·0	58·4	31·3	30·4
J	59·0	57·7	30·9	30·3

* Each result is the mean of four determinations.
† Results supplied by Beecham Pharmaceuticals.

The maximum difference in absorbance of ampicillin is obtained by using buffers of pH less than 5 and greater than 9 (Fig. 2). Buffers of pH 5 and pH 9 were chosen for the assay of ampicillin because of the known instability of penicillins in highly acidic or alkaline solutions.[1] Provided that the assay is carried out within 30 minutes of the preparation of the solutions, the hydrolysis of the penicillins is negligible (Table III).

It is important that measurement of absorbance differences at 268 nm is carried out in cells of matched path lengths. Even a slight difference in path length will produce an error in the absorbance difference of the sample solution due to the high absorbance of the cloxacillin. Thus, there should be zero absorbance at 268 nm when a solution of cloxacillin sodium in water (0·08 per cent. *m/V*) is placed in both the reference and test cells.

The concentration of cloxacillin is obtained after correction of the total absorbance of the sample at 275 nm for the absorbance of the ampicillin. The contribution of ampicillin to the total absorbance at this wavelength is small, and it has been calculated that, for a mixture of equal parts of ampicillin and cloxacillin, an error of 10 per cent. in the calculated ampicillin concentration results in an error of only 0·5 per cent. in the calculated concentration of cloxacillin.

In the microbiological assay of ampicillin and cloxacillin, the limits of error for a single determination are about ±7·0 per cent. for ampicillin and ±5·5 per cent. for cloxacillin (D. Sykes, personal communication). As the limits of error for a single determination ($P = 0·95$) in the present work ranged only from ±1·81 per cent. for ampicillin to ±3·66 per cent. for cloxacillin (Table II) greater precision is obtained with the spectrophotometric method.

Of the penicillins examined (Table IV), only ampicillin and amoxycillin exhibit absorbance differences at 268 nm. Amoxycillin (2-(−)-amino-*p*-hydroxybenzylpenicillin) shows a large, negative absorbance difference at 268 nm, probably due to ionisation of the phenolic group in alkaline solution. Pivampicillin, the pivaloyloxymethyl ester of ampicillin, precipitates in pH 9 buffer and therefore cannot be determined by the procedure. Other penicillins, lacking an α-aminobenzyl side chain substituent, gave a zero absorbance difference at 268 nm and it should be possible to determine ampicillin in the presence of these penicillins. However, the absorbances (1 per cent., 1 cm) of phenethicillin, phenoxymethylpenicillin, methicillin, propicillin and flucloxacillin at 268 nm are two to four times higher than that of cloxacillin. In order to avoid the high noise level in the measurement of the absorbance difference of ampicillin, the amount of mixed penicillins used in the assay would have to be reduced, with consequent loss in precision.

Ampicillin sodium and ampicillin trihydrate (dissolved by the addition of a few drops of pH 9 buffer solution) show equal absorbance differences on an equimolar basis (Table IV). Standard mixtures of ampicillin trihydrate and cloxacillin sodium analysed by the procedure gave good results; however, commercial mixtures of these penicillins, formulated as a dry powder for suspension, gave good results for ampicillin but high results for cloxacillin, due to absorption at 275 nm by other components of the suspension.

Ampicillin and the penicilloic acid formed by penicillinase hydrolysis of ampicillin gave equal absorbance differences at 268 nm (Table IV). The results for ampicillin will therefore include any ampicillin penicilloic acid present in the mixture. The levels of ampicillin penicilloic acid are low at the time of manufacture but may increase on storage. The extent of degradation in old samples may be quickly determined by measurement of their iodine consumption[6] and samples containing unacceptable levels of penicilloic acids should be assayed by the more specific, but less precise, gel electrophoretic method.

The results obtained by use of the present method for standard mixtures are in good agreement with the theoretical values (Table I). The results obtained for commercial samples are in reasonable agreement with those obtained by the manufacturer using microbiological assay after separation of the penicillins by gel electrophoresis (Table V).

The principal advantages of the present method are that it is rapid, as preliminary separation of the penicillins is unnecessary, and that it is more precise than the microbiological method of assay.

We thank Beecham Pharmaceuticals for supplying materials and the microbiological results and Mrs. M. Tweedie for technical assistance.

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Received *August 20th*, 1973
Accepted *March 7th*, 1974

Colorimetric Determination of Piperazine in Pharmaceutical Formulations

By YEHIA M. DESSOUKY

(*Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt*)

AND SAAD A. ISMAIEL

(*Research Department, Société Misr pour l'Industrie Pharmaceutique, 92 El Mataria Street, Post El Zeitoun, Cairo, Egypt*)

Piperazine can be satisfactorily determined in pharmaceutical preparations or formulations such as effervescent granules and elixirs containing hexamine, colchicine, atropine sulphate, sodium benzoate, lithium benzoate, lithium citrate, sodium citrate, sodium hydrogen carbonate, tartaric acid, citric acid, lactose, sucrose and Tinct. ammi visnaga. The diluted sample solution is treated with a 0·6 per cent. aqueous 1,2-naphthoquinone-4-sulphonate solution in the presence of acetate - citrate buffer at pH 7·5. The temperature of the reaction should be between 10 and 15 °C and the colour produced is measured at 490 nm.

Piperazine, a pyrazine derivative, is one of the most potent drugs used as an anthelmintic for the treatment of threadworms and roundworms in both children and adults, the worms usually being voided from the host while the drug is still active.

Piperazine in effervescent granules cannot be determined by using either the non-aqueous titration method of the United States Pharmacopeia[1] or the gravimetric method of the British Pharmacopoeia.[2] Further, the colorimetric methods of Pankratz,[3] who used the reineckate salt, and Perlmutter,[4] who used acidified *p*-benzoquinone, could not be applied to this preparation. This inapplicability is because of the presence and interference of hexamine, citric and tartaric acids, sodium citrate, sodium hydrogen carbonate and other interfering ingredients that yield either higher or lower results. The titrimetric formol method suggested by Simionovici, Răianu and Cuculescu[5] also cannot be used for an accurate determination of piperazine in coloured pharmaceutical preparations as the colour masks the end-point, giving high results. Gravimetric methods, such as those described by Maynard[6] for the diacetyl derivative and by Chemerisskaya[7] for the dichromate derivative were tried, but accurate results were not obtained as the methods are not sensitive. They are time consuming, however.

In the present investigation, a colorimetric method for the determination of piperazine in some pharmaceutical preparations is proposed that involves the use of the red colour formed on treating piperazine with 1,2-naphthoquinone-4-sulphonate in the presence of acetate - citrate buffer at pH 7·5. The proposed method is free from the drawbacks of the above methods.[1–7]

Experimental

Reagents—

The pure piperazine used was of pharmaceutical grade and all of the chemicals and reagents were either of analytical-reagent or pharmaceutical grade.

1,2-Naphthoquinone-4-sulphonate reagent—Dissolve 0·3 g of 1,2-naphthoquinone-4-sulphonate sodium salt in 50 ml of water. This reagent must be freshly prepared.

Acetate - citrate buffer, pH 7·5—Dissolve 5·0 g of sodium acetate and 1·0 g of sodium citrate in about 50 ml of water and adjust the pH of the solution to 7·5 with a few drops of 33 per cent. acetic acid, then dilute to a volume of 100 ml with water and filter if necessary.

Standard solution—Weigh accurately 100 mg of piperazine citrate (or hexahydrate, depending on the formulation) and dissolve it in sufficient water to produce 100 ml. Dilute 3 ml of this solution to 100 ml with water.

Samples—The composition of each of the samples tested is given in Table I.

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TABLE I

FORMULATION OF ANALYSED SAMPLES

Constituent	Sample I	II	III	IV	V
Piperazine citrate/g	14·0	1·5	—	1·125	—
Piperazine hexahydrate/g	—	—	1·8	—	0·9
Hexamine/g	—	5·0	—	1·5	4·5
Colchicine/g	—	—	4·2	—	—
Atropine sulphate/g	—	—	1·8	—	—
Sodium benzoate/g	—	—	—	2·25	—
Lithium benzoate/g	—	—	—	1·5	—
Lithium citrate/g	—	—	—	—	6·3
Sodium citrate/g	—	—	9·6	—	—
Sodium hydrogen carbonate/g	—	12·5	—	30·65	46·8
Tartaric acid/g	—	—	—	16·2	23·4
Citric acid/g	—	10·0	—	10·3	23·4
Lactose/g	—	—	—	3·0	4·5
Sucrose/g	—	—	—	8·025	—
Tinct. ammi visnaga/ml	—	17·0	—	—	—
Coloured flavoured syrup/ml	to 100·0	—	—	—	—
Flavoured, coloured effervescent mixture/g	—	to 50·0	—	—	—
Effervescent mixture/g	—	—	to 70·0	to 90·0	to 90·0

Samples I and II (Vermizine elixir and coli-urinal effervescent granules) supplied by Société Misr pour l'Industrie Pharmaceutique, Cairo, Egypt.

Sample III (Urosolvine effervescent granules) supplied by the Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt.

Sample IV (Ciluryl effervescent granules) supplied by ADCO (The Arab Drug Company), Cairo, Egypt.

Sample V (Urolithine effervescent granules) supplied by Kahira Pharmaceutical and Chemical Industries, Cairo, Egypt.

PREPARATION OF SAMPLE SOLUTIONS—

For effervescent granules, transfer an amount of the sample equivalent to 100 mg of piperazine citrate (or hexahydrate, depending on the formulation) into a 100-ml calibrated flask, add about 50 ml of water, and when the effervescence has ceased, dilute to volume with water. Dilute 3 ml of this solution to 100 ml with water.

For elixirs, dilute the appropriate volume of the sample with water to give a final concentration equivalent to 3 mg of piperazine citrate (or hexahydrate, depending on the formulation) in 100 ml of water.

TABLE II

COMPARISON BETWEEN THE RESULTS OF THE PROPOSED METHOD AND THE B.P. 1968 METHOD

	Amount of piperazine citrate (or hexahydrate)					
			Recovered‡ by proposed method		Recovered‡ by B.P. 1968 method	
Sample*	Taken/μg	Standard added†/μg	μg	per cent.	μg	per cent.
I	60	—	64·7	107·8	48·0	80·0
	—	60	60·3	100·5	—	—
II	60	—	62·5	104·1	38·4	64·0
	—	60	60·0	100·0	—	—
III	60	—	59·4	99·0	50·1	83·5
	—	60	60·8	101·3	—	—
IV	60	—	56·3	93·8	57·6	96·0
	—	60	60·3	100·5	—	—
V	60	—	58·2	97·0	§	—
	—	60	61·0	101·6		

The standard deviation is ±0·97 per cent. for the proposed method.

* See footnote to Table I.

† Added as a 0·003 per cent. aqueous solution of piperazine citrate (or hexahydrate).

‡ Each value given is the average of three experiments.

§ The precipitated piperazine picrate was unfilterable.

PROCEDURE—

To 2 ml of the standard and sample solutions in separate test-tubes, add 5 ml of the acetate - citrate buffer, pH 7·5, mix well and then cool the mixtures in a water-bath at a temperature between 10 and 15 °C for 2 minutes. Add to each test-tube 3 ml of 1,2-naphthoquinone-4-sulphonate reagent, again mix well and leave them to stand for about 10 minutes. Measure the absorbance of both standard and sample solutions at a wavelength of 490 nm against a blank carried out simultaneously. A Carl Zeiss, Model M4 QII, spectrophotometer was used.

Calculate the amount of piperazine citrate (or piperazine hexahydrate) as follows:

$$\frac{T}{S} \times \frac{m_1}{m_2} \times 100 = \text{piperazine salt in sample, per cent. } m/m$$

where T and S are the absorbance values of sample and standard, respectively, m_1 is the amount of standard present in 2 ml, and m_2 is the amount of sample present, theoretically, in 2 ml. The results are given in Table II.

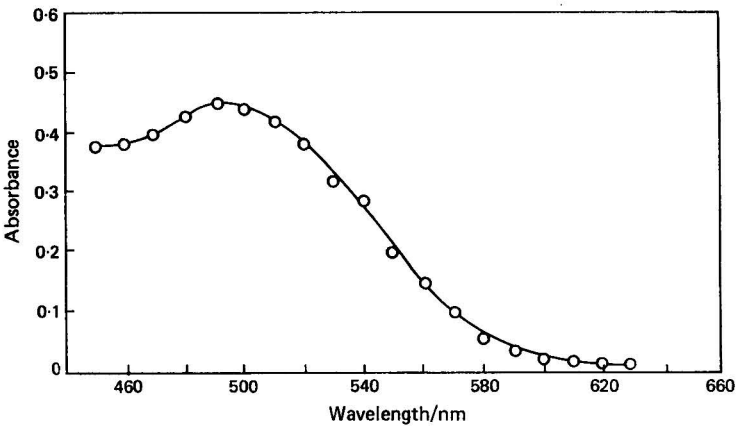

Fig. 1. Light absorbance spectrum of the colour formed

RESULTS AND DISCUSSION

During the study of the reaction of 1,2-naphthoquinone-4-sulphonate with some secondary amines, a red colour was observed on treating piperazine with 1,2-naphthoquinone-4-sulphonate at a pH between 6 and 9.

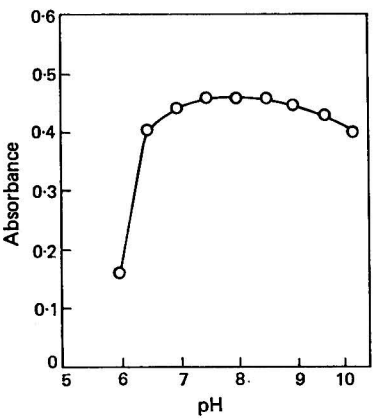

Fig. 2. Optimum pH for the reaction (wavelength, 490 nm)

A study of the colour formed showed that the maximum absorption occurs at 490 nm (Fig. 1), that the optimum pH for the reaction is 7·5 (Fig. 2) and that the optimum concentration of the reagent is 18 mg per 10 ml (Fig. 3).

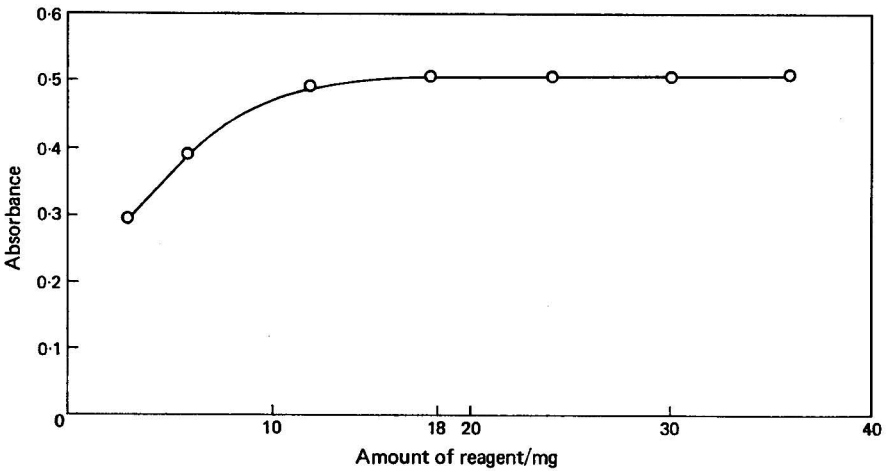

Fig. 3. Variation of absorbance with amount of 1,2-naphthoquinone-4-sulphonic acid, sodium salt (wavelength, 490 nm)

The coloured product of the reaction started to precipitate after about 15 minutes. This time decreased with increase in the concentration of piperazine (Fig. 4). The above precipitation accounts for the slight differences in the calibration graphs.

The addition of not only ethanol, but also methanol, propan-1-ol and propan-2-ol, was tried, without success in reducing the differences. Extraction into an immiscible solvent, such as chloroform, was also tried and gave partial extraction. However, the organic layer changed to a yellowish colour. The stability of the red colour (before the formation of a red precipitate) differs according to the concentration. The most stable colour is obtained when 60 μg of piperazine salt are used (Fig. 4).

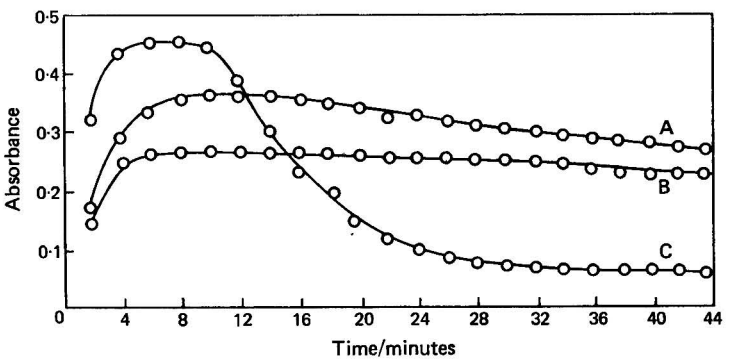

Fig. 4. Colour stability at different concentrations of piperazine salt (wavelength, 490 nm): A, 80 μg; B, 60 μg; and C, 100 μg

Beer's law is obeyed for amounts of piperazine citrate from 20 to 120 μg (Fig. 5), although the simultaneous use of a standard, with which to compare the sample, is found to be necessary in order to obtain accurate results, as the slope of the calibration graph differs slightly on repetition.

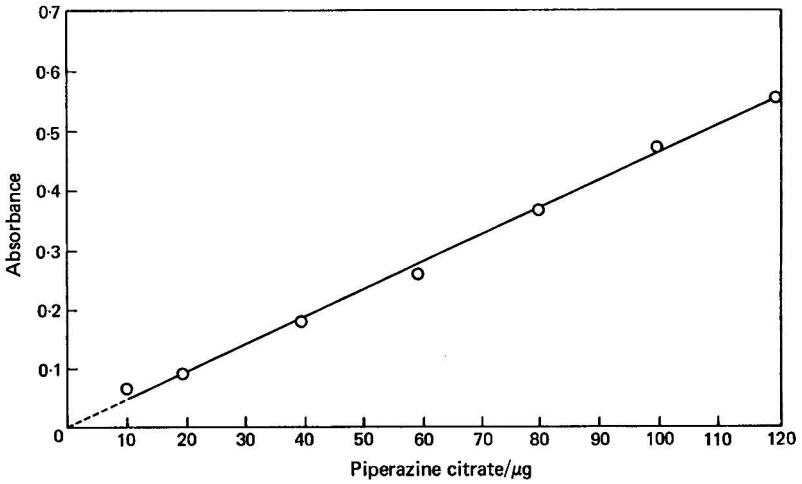

Fig. 5. Calibration graph for different amounts of piperazine citrate (wavelength, 490 nm)

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Received *April 19th*, 1973
Accepted *February 4th*, 1974

Analyst, August, 1974, Vol. 99, *pp.* 487–490

Colorimetric Determination of Antazoline in Some Pharmaceutical Preparations with Sodium Nitrite

By M. M. AMER, M. S. TAWAKKOL

(Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt)

AND S. A. ISMAIEL

(Research Department, Société Misr pour l'Industrie Pharmaceutique, 92 El Mataria Street, Post El Zeitoun, Cairo, Egypt)

A colorimetric method for the determination of antazoline in pharmaceutical preparations with sodium nitrite has been developed. The method involves treatment of a cooled and acidified dilute aqueous solution of the sample with sodium nitrite. The yellow colour produced is stabilised by the addition of propan-2-ol or ethanol and the absorbance then measured at 410 nm.

Naphazoline, tolazoline, clemizole, diphenhydramine, chlorpheniramine, ephedrine, cetrimide, benzalkonium chloride and zinc salts, even if present in amounts ten times greater than that of antazoline, do not interfere.

ANTAZOLINE can be determined in some pharmaceutical preparations by using the acidimetric methods of the B.P.,[1] the B.P.C.,[2] the non-aqueous titration methods of the U.S.P.,[3] Selles and Rodriguez[4] and Rink and Riemhofer[5] or by using the $E^{1\%}_{1cm}$ value mentioned by Clarke,[6] but none of these methods can be used if antazoline occurs in combination with ephedrine, diphenhydramine or chlorpheniramine.

Phenylephrine, if present, interfered in the determination by the last four of the above methods, while naphazoline interfered in the determination by the methods of Slack and Mader,[7] Horioka and Ishioka[8] and Kum-Tatt,[9] giving high results.

In this paper, a sensitive colorimetric method for the determination of antazoline in pharmaceutical preparations is proposed in which use is made of the qualitative colour reaction reported by Auterhoff.[10] The proposed method was found to be simple, quick and devoid of the drawbacks of the methods mentioned above.

EXPERIMENTAL

REAGENTS—

Antazoline hydrochloride standard stock solution—Dissolve 100 mg of antazoline hydrochloride in sufficient water to give a final volume of 100 ml.

Antazoline methanesulphonate standard stock solution—Dissolve an amount of antazoline methanesulphonate (previously determined according to the B.P.C. 1968) in sufficient water to give a final concentration of 100 mg of antazoline methanesulphonate per 100 ml.

Sodium nitrite solution—Dissolve 2 g of sodium nitrite (Merck) in sufficient water to give a final volume of 100 ml.

Hydrochloric acid, approximately 2·5 N.

Hydrochloric acid, concentrated.

Ethanol, 95 *per cent.*

CALIBRATION GRAPH—

Dilute the appropriate volume of the antazoline salt stock solution with water to give solutions of final concentrations ranging between 0·4 and 2·4 mg of the antazoline salt per 100 ml.

Transfer 5 ml of the diluted antazoline salt solution (containing 20 to 120 μg of the corresponding antazoline salt) into a glass-stoppered test-tube, cool it in ice, add 2 ml of 2·5 N hydrochloric acid, mix and then add 1 ml of sodium nitrite. Mix the solution and, after

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2 minutes, add 2 ml of ethanol, mix again, then remove the test-tube from the ice and, after 3 minutes, measure, at 410 nm, the absorbance of the yellow colour produced against a blank carried out simultaneously.

PREPARATION OF SAMPLE SOLUTIONS—

Tablets—Transfer an amount of the powder containing about 50 mg of antazoline hydrochloride into a 100-ml calibrated flask, add about 80 ml of water and shake the flask until the powder has dissolved (about 30 minutes). Dilute this solution to volume with water, filter it through a dry Whatman No. 1 filter-paper and dilute 2 ml of it to 100 ml with water.

Drops, injectable solutions and syrups—Dilute an appropriate volume of the sample with water so as to give a final concentration of about 1 mg of the antazoline salt per 100 ml.

Creams—Transfer an amount of the cream containing about 100 mg of antazoline hydrochloride into a beaker and extract it successively with 50, 20 and 20 ml of 0·5 N hydrochloric acid by heating the mixture each time on a boiling water bath, while stirring, until the cream has melted, allowing it to stand for about 5 minutes, then cooling it in ice, and decanting the aqueous extract into a separating funnel.

Extract the combined aqueous extracts with about 10 ml of chloroform, make the aqueous layer up to 200 ml with water, then dilute 2 ml of the latter to 100 ml with water.

Lotions—Transfer 5 ml of the well shaken sample into a 100-ml calibrated flask, add 5 ml of concentrated hydrochloric acid, mix and, when the effervescence ceases, dilute the mixture to volume with water. Filter it through a dry Whatman No. 1 filter-paper and reject the first turbid 10 ml of the filtrate, then dilute a volume containing about 1 mg of antazoline hydrochloride to 100 ml with water.

DEVELOPMENT OF THE COLOUR—

Transfer 5 ml of the prepared sample solution into a glass-stoppered test-tube and complete the procedure as directed under Calibration graph, starting with "cool it in ice, add 2 ml of 2·5 N hydrochloric acid . . ."

Calculate the concentration of the antazoline salt from the calibration graph.

RESULTS AND DISCUSSION

The results obtained by applying the proposed method to the determination of antazoline hydrochloride and antazoline methanesulphonate in some pharmaceutical preparations are given in Table I.

TABLE I

COMPARISON OF RESULTS BY THE PROPOSED METHOD AND SLACK AND MADER'S METHOD

Pharmaceutical preparations*	Antazoline salt			
	Indicated on label/mg	Added to labelled material/mg	Recovery, per cent.†	
			by proposed method	by Slack and Mader's method
Sensol tablets	100	—	99·6 ± 1·06	103·04 ± 1·34
		50	100·1 ± 0·65	—
Antistine ampoules	50	—	100·4 ± 1·14	101·5 ± 1·89
		25	100 ± 0·81	—
Antistine privine solution	500	—	98·42 ± 2·32‡	112 ± 2·52‡
		250	98·93 ± 1·35	—
Fenozal drops§	500	—	100·92 ± 0·61	101·92 ± 0·31
Calazol lotion	1000	—	95·6 ± 1	not applicable
		500	97·4 ± 0·82	
Calazol cream	2000	—	105 ± 2·37	not applicable
		1000	103·83 ± 1·82	

* Sensol tablets, fenozal drops, calazol lotion and calazol cream were supplied by Société Misr pour l'Industrie Pharmaceutique, Cairo, Egypt. Antistine ampoules and antistine privine solution were supplied by Ciba Laboratories, Basle, Switzerland.

† Mean of six experiments.

‡ Calculated as antazoline hydrochloride; for conversion into antazoline sulphate results were multiplied by 1·041.

§ Specially prepared authentic sample containing the correct amount of ingredients.

The qualitative colour reaction of antazoline and sodium nitrite reported by Auterhoff[10] was studied and used in order to develop a quantitative method for the determination of antazoline in pharmaceutical preparations.

The absorption spectrum of the colour obtained showed a maximum at 410 nm (Fig. 1) and the maximum intensity of the colour was obtained by using 20 mg of sodium nitrite (Fig. 2) and hydrochloric acid of about 2·5 N concentration (Fig. 3) (both sulphuric and hydrochloric acids at about 2·5 N concentration gave the same colour intensity). The colour reached its maximum intensity after about 10 minutes, remained stable for about 5 minutes and then began to fade (Fig. 4).

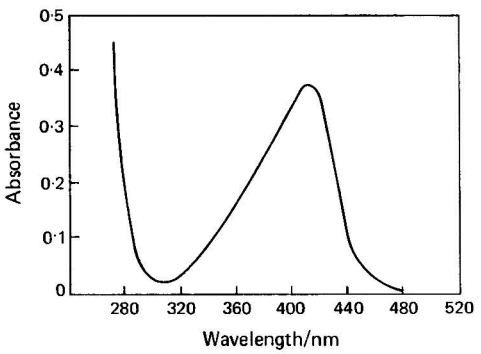

Fig. 1. Absorption spectrum of the colour produced

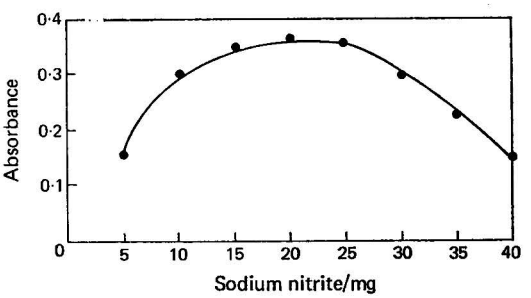

Fig. 2. Effect of the amount of sodium nitrite on the intensity of the colour

The use of ethanol (or propan-2-ol) was found to improve the stability of the colour (Fig. 4), the intensity of which obeyed Beer's law for amounts of antazoline hydrochloride between 20 and 120 μg and of antazoline methanesulphonate between 20 and 140 μg.

The colour was found to be unstable at room temperature or on heating the solution but cooling the latter in ice or to a temperature below 10 °C improved the stability of the colour (Fig. 4).

As shown in Table I, the proposed method gave more accurate results than those obtained by the method of Slack and Mader, especially for samples that contain naphazoline.

Diphenhydramine, chlorpheniramine, naphazoline, ephedrine, codeine, benzalkonium chloride, phenylephrine, tolazoline, clemizole, phenol, camphor, esters of *p*-hydroxybenzoic acid, sodium cyclamate, saccharin sodium, zinc oxide and calamine in amounts ten times greater than that of antazoline did not interfere.

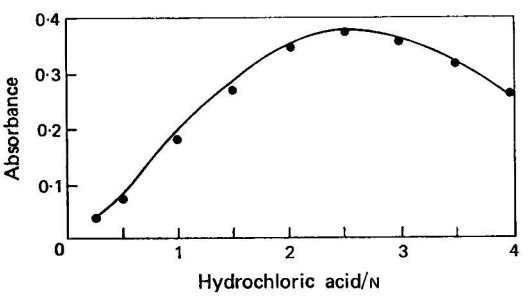

Fig. 3. Effect of concentration of hydrochloric acid on the intensity of the colour

The reaction is probably due to the aniline moiety of the antazoline molecule, because naphazoline and tolazoline, which contain the same imidazoline ring but no aniline moiety, did not give a colour, whereas phentolamine and diethylaniline, which contain an aniline moiety, gave a yellow colour with a maximum absorption at 360 nm.

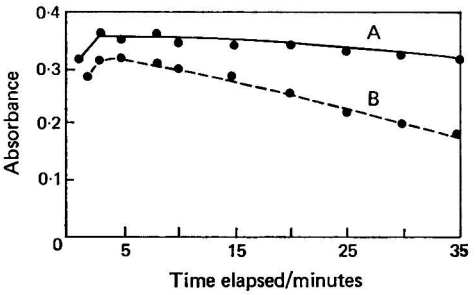

Fig. 4. Improved stability of the colour at temperatures below 10 °C (solutions cooled in an ice-cold water-bath for both stable and unstable colours): A, ethanol - water; and B, water

The explanation that the colour results from the formation of a nitroso derivative of antazoline similar to that which occurs in the nitrosation of a dialkylaniline such as diethylaniline with nitrous acid, as stated by Finar[11,12] and Mann and Saunders,[13,14] cannot be supported as the maximum absorption of the yellow colour produced with phentolamine and diethylaniline differed greatly from that with antazoline.

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Received *December 17th*, 1973
Accepted *February 25th*, 1974

Analyst, August, 1974, Vol. 99, *pp.* 491–497

Colorimetric Determination of Small Amounts of C_8 to C_{10} Alcohols in Their Phthalate Esters

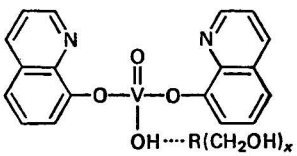
BY S. HARRISON, H. HINCHCLIFFE AND G. L. WOODROFFE

(*Research and Development Department, Imperial Chemical Industries Limited, Petrochemicals Division, Billingham, Teesside, TS23 1JB*)

Methods were examined for the determination of small amounts of C_8 to C_{10} alcohols in their phthalate esters involving the use of vanadium 8-hydroxyquinolinate in benzene or toluene and 3,5-dinitrobenzoyl chloride in pyridine as colorimetric reagents. Two modified procedures are presented that will reliably determine free alcohols in the 0·01 to 0·4 per cent. *m/m* range.

PHTHALATE esters are widely used as plasticisers and the presence of even small amounts of free alcohols can lead to undesirable odours in the finished plastic. Reliable and sensitive analytical methods are therefore required in order to monitor the residual alcohol content at levels of 0·01 to 0·5 per cent. The alcohols commonly used are both straight- and branched-chain alcohols of carbon number 8 to 10.

Buscarons, Marin and Claver[1] were the first to use vanadium 8-hydroxyquinolinate (oxinate) for detecting small amounts of alcohols. Feigl and Stark[2] used the reagent as a spot test for primary, secondary and tertiary alcohols. It is suggested that the colour complex formed is



Following this work, vanadium 8-hydroxyquinolinate has been widely used for determining alcohols.[3–17] Poor reproducibility and sensitivity and in some instances the unpleasant or toxic nature of the solvents used have made the published methods unsuitable for the above application.

An alternative reagent for the determination of alcohols is 3,5-dinitrobenzoyl chloride, the use of which was developed by Berezin[18] and extended by Johnson and Critchfield.[19] The reaction of 3,5-dinitrobenzoyl chloride with alcohols in the presence of pyridine gives products that are highly coloured in some non-aqueous media. This reaction has been applied to both the identification and the determination of alcohols,[20–22] including C_8 to C_{18} alcohols in their fumaric esters.[14]

The purpose of this paper is to give the experimental background and the details of analytical procedures involving the use of vanadium 8-hydroxyquinolinate and 3,5-dinitrobenzoyl chloride that have been found to be satisfactory for the determination of free alcohols (C_8 to C_{10}) down to 100 p.p.m. in phthalate esters. A standard procedure for preparing vanadium 8-hydroxyquinolinate is also described.

VANADIUM 8-HYDROXYQUINOLINATE METHOD

REAGENTS—

Glacial acetic acid, 2·5 per cent. V/V solution in toluene.

Dichloroacetic acid, 10 per cent. V/V solution in glacial acetic acid.

Toluene, alcohol-free—Wash 1 litre of toluene with 100 ml of 1 N sulphuric acid containing 1 g of potassium chromate, followed by 100 ml of 1 N sodium hydroxide solution, then wash the toluene with four 100-ml volumes of distilled water and dry it over anhydrous sodium sulphate.

Sodium hydroxide solutions, 1 N and 0·1 N.

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Dimethylformamide.

Standard solution of alcohol in toluene—

1 ml of solution ≡ 0·2 mg of alcohol (*e.g.*, "isodecanol"* for diisodecyl phthalate).

Sodium salt of vanadium 8-hydroxyquinolinate—Dissolve 1·35 g of ammonium metavanadate in 35 ml of 1 N sodium hydroxide solution in a 250-ml beaker. Add 15 ml of distilled water and boil the mixture for 10 minutes in order to remove all the ammonia, adding water as required to keep the volume at 50 ml. This solution (solution A) is maintained at its boiling-point.

Dissolve 5 g of 8-hydroxyquinoline in 35 ml of hot 1 N sodium hydroxide solution in another 250-ml beaker. Add 40·0 ml of distilled water, bring the solution to its boiling-point, then place the beaker in a boiling water bath. Add slowly, with stirring, the hot solution A and allow the beaker to stand in the bath for 15 to 20 minutes. The volume of the solution should be kept constant at 125 ml by adding hot distilled water. Remove the beaker from the bath, place it on top of the bath and add 50 per cent. *V*/*V* acetic acid dropwise from a burette, stirring the solution vigorously during the addition. A black precipitate forms on addition of acetic acid, which readily coagulates and also partly re-dissolves in the alkaline solution. The supernatant solution remains clear after the addition of each drop of acetic acid and coagulation of the black precipitate. Continue to add acetic acid dropwise until the solution becomes cloudy and remains cloudy after stirring without further addition of acetic acid (approximately 6 ml of 50 per cent. *V*/*V* acetic acid are required to neutralise the excess alkalinity; the pH of the solution should be 7 to 8).

Quickly filter the hot solution through a Whatman No. 541 filter-paper and cool the filtrate in an ice-bath until a copious yellow precipitate of the sodium salt of vanadium 8-hydroxyquinolinate is deposited. Filter off the yellow precipitate using a vacuum filter. If necessary, wash out the flask with not more than 5 ml of distilled water in order to transfer all the yellow precipitate to the filter. (Do not use more than this volume of water to wash the precipitate as it is very soluble in water.) Continue vacuum filtration until all the excess of liquor has been sucked from the precipitate. Remove the yellow precipitate and the filter-paper from the funnel and place it on a large watch-glass. Dry the precipitate in an oven at 80 °C for 4 hours; the yellow solid will then be sufficiently dry to remove the filter-paper and also to be broken down into a coarse powder. Replace the precipitate in the oven for a further 24 hours at 80 °C. Grind the precipitate to a fine powder and store it in a glass container until required.

Vanadium 8-hydroxyquinolinate reagent—Weigh 0·2 g of the sodium salt of vanadium 8-hydroxyquinolinate into a 150-ml conical flask, add 15 ml of dimethylformamide and shake the flask so as to dissolve the solid. Add 100 ml of alcohol-free toluene, mix, then add 10 ml of 2·5 per cent. *V*/*V* acetic acid in toluene, and mix again. This procedure should give a dark solution. Prepare the reagent freshly as required; it should be used the same day.

Apparatus—

A Unicam SP600 spectrophotometer or another suitable colorimeter is used.

Procedure—

Weigh a suitable amount of sample into a 25-ml calibrated flask, dilute to the mark with alcohol-free toluene and mix well. Take three 25-ml measuring cylinders, A_1, B_1 and C_1.

Into A_1 and B_1 transfer by pipette 5-ml aliquots of diluted sample and to A_1 add 2 ml of standard alcohol solution. To B_1 add 2 ml and to C_1 add 7 ml of alcohol-free toluene; the volume of liquid in each cylinder is now 7 ml. Add 10 ml of vanadium 8-hydroxyquinolinate reagent to each cylinder, stopper, mix, then heat them to 60 ± 1 °C for 20 minutes in a water-bath. Cool the cylinders in cold water for 2 minutes, then add 10 ml of 0·1 N sodium hydroxide solution to each and stopper and shake them vigorously for 1 minute. If phenolic antioxidants are present, the dark colour remains, otherwise the solution becomes pink to red. All reagents and the sample must be dry (*i.e.*, give clear solutions in toluene) up to the stage of the addition of the 0·1 N sodium hydroxide solution.

Allow the layers to separate, then transfer by pipette 5 ml of the upper toluene layer from each cylinder into each of three 10-ml stoppered measuring cylinders, A_2, B_2 and C_2. Add 1 ml

* "Isodecanol" is a mixture of branched-chain decyl alcohols.

of dichloroacetic acid reagent to each and shake to mix. Measure the absorbance at 620 nm in 10-mm cells using the solution in C_2 as a blank.

CALCULATION—

Let M g be the mass of sample, a mg the mass of alcohol added as internal standard, *i.e.*, the mass of alcohol added to cylinder A_1, A_A the absorbance of the solution in A_2 against that in C_2 and A_B the absorbance of the solution in B_2 against that in C_2. Then,

$$\text{Free alcohol*, per cent. } m/m = \frac{A_B \times a \times 25 \times 100}{(A_A - A_B) \times M \times 5 \times 1000}$$

DINITROBENZOYL CHLORIDE METHOD

REAGENTS—

3,5-Dinitrobenzoyl chloride reagent—Dissolve 1 g of 3,5-dinitrobenzoyl chloride in 10 ml of dry pyridine (containing less than 0·05 per cent. m/V of water), warming the mixture on a steam-bath to assist solution. Prepare the reagent immediately before use; it will keep for approximately 1 hour.

Pyridine—Analytical-reagent grade. The water content must be less than 0·05 per cent. m/V (it must be specially dried).

Cyclohexane or n-hexane—Analytical-reagent grade.

Hydrochloric acid—AnalaR grade.

Sodium carbonate—AnalaR grade. Prepare a 5 per cent. m/V solution.

1,2-Diaminopropane.

Dimethylformamide—Analytical-reagent grade. The water content must be less than 0·5 per cent. m/V.

Standard alcohol solution—Prepare a 4 per cent. m/V solution in dry pyridine.

APPARATUS—

A Unicam SP600 spectrophotometer or another suitable colorimeter is used. A 10-μl Hamilton syringe is required.

PROCEDURE—

Weigh a suitable amount of sample into a 25-ml calibrated flask, dilute to the mark with dry pyridine and mix well. Take three 150-ml Erlenmeyer flasks, A_1, B_1 and C_1. Into A_1 and B_1 transfer by pipette 2-ml aliquots of the sample solution and into C_1 2 ml of dry pyridine. Into A_1 inject 10 μl of the standard alcohol solution with the Hamilton syringe and then into all three flasks transfer by pipette 1-ml aliquots of the 3,5-dinitrobenzoyl chloride reagent.

Stopper the flasks and mix the contents well. Allow the flasks to stand for 15 minutes, then add 25 ml of approximately 2 N hydrochloric acid to each flask and transfer the contents into each of three separating funnels. Rinse out each flask with 20 ml of cyclohexane and transfer the rinsings into the appropriate separating funnel. Stopper the separating funnels and shake each vigorously for 1 minute, then allow the contents to settle.

Run off the bottom aqueous phase from each separating funnel, then add 5 ml of 5 per cent. m/V sodium carbonate solution to each, shake them for approximately 30 s, then allow the contents to settle. Run off the sodium carbonate solution from each separating funnel and filter the cyclohexane phase through a small cotton-wool plug contained in a filter funnel into each of three 25-ml stoppered measuring cylinders, A_2, B_2 and C_2. Wash the cotton-wool plugs with sufficient fresh cyclohexane to make the volume in each cylinder up to 25 ml, then mix the contents of the cylinders well.

Transfer by pipette 1 ml of the cyclohexane solution from each measuring cylinder into each of three 10-ml stoppered measuring cylinders, A_3, B_3 and C_3. Add 5 ml of dimethylformamide and 1 ml of 1,2-diaminopropane to each cylinder and mix the contents well, then, after 5 to 10 minutes, measure the absorbances of the solutions in A_3 and B_3 at 525 nm in 10-mm cells using the solution in C_3 as a blank.

* Calculated as the alcohol component of the ester.

CALCULATION—

Let M g be the mass of sample, a mg the mass of alcohol added as internal standard, *i.e.*, the mass of alcohol added to flask A_1, A_A the absorbance of the solution in A_3 against that in C_3 and A_B the absorbance of the solution in B_3 against that in C_3. Then,

$$\text{Free alcohol*, per cent. } m/m = \frac{A_B \times a \times 25 \times 100}{(A_A - A_B) \times M \times 2 \times 1000}$$

TABLE I

EFFECT OF DRYING THE SODIUM SALT OF VANADIUM 8-HYDROXYQUINOLINATE ON THE ABSORBANCE DUE TO 2-ETHYLHEXANOL

Drying procedure	2-Ethylhexanol/mg	Absorbance
Dried in vacuum desiccator	0·20	0·07
	0·40	0·14
	0·60	0·22
	0·80	0·31
Dried at 60 °C	0·20	0·11
	0·40	0·22
	0·60	0·32
	0·80	0·45
Dried at 80 °C	0·20	0·12
	0·40	0·24
	0·60	0·35
	0·80	0·47

TABLE II

EFFECT OF REACTION TIME ON THE ABSORBANCE DUE TO ISOOCTANOL

Time in water-bath at 55 °C/minutes	0	2	5	10	15	20	30	60
Absorbance at 620 nm	0·26	0·39	0·49	0·52	0·53	0·52	0·52	0·51

TABLE III

STABILITY OF THE BLUE COMPLEX FORMED WITH VANADIUM 8-HYDROXYQUINOLINATE

The colour was developed on a solution of isooctanol and the absorbance at 620 nm was measured at intervals

Time/minutes	2	5	10	15	20	30	60	180
Absorbance	0·44	0·44	0·43	0·43	0·42	0·41	0·40	0·39

TABLE IV

STABILITY OF VANADIUM 8-HYDROXYQUINOLINATE REAGENT

Time of test/hours	Absorbance†	
	In benzene	In toluene
0	0·40	0·24
6	—	0·17
24	0·20	0·06

† Isooctanol was used as the alcohol and the absorbance was measured at 620 nm.

TABLE V

EFFECT OF WATER IN PYRIDINE ON THE REACTION OF 3,5-DINITROBENZOYL CHLORIDE WITH ISOOCTANOL

Concentration of water in pyridine, per cent. m/m	Mass of isobutanol	Absorbance
0·02	0·7	0·35
0·12	0·7	0·19
0·15	0·7	0·12
0·20	0·7	0·10
0·25	0·7	0·08

* Calculated as the alcohol component of the ester.

TABLE VI

STABILITY OF THE COLOURED COMPLEX FORMED BETWEEN 3,5-DINITROBENZOYL CHLORIDE AND ALCOHOLS

	Absorbance			
Time/minutes	Sample 1	Sample 2	Sample 3	Sample 4
3	0·27	0·36	0·57	0·65
10	0·26	0·36	0·56	0·63
30	0·23	0·30	0·50	0·56

RESULTS AND DISCUSSION

VANADIUM 8-HYDROXYQUINOLINATE METHOD—

Benzene was originally used to replace nitrobenzene as solvent in this method because it gave increased sensitivity, but was unsatisfactory owing to its toxicity. Of the various solvents tried, *viz.*, toluene, xylenes, cyclohexane and n-heptane, the most suitable was toluene, although the sensitivity obtained was only about 60 per cent. of that obtained with benzene.

Examination of the problems involved in preparing vanadium 8-hydroxyquinolinate reagent satisfactorily revealed ambiguities in the procedural details of preparation. It was also found that small amounts of water in the reagent had an adverse effect on sensitivity. These problems were resolved and a preparation procedure was devised that included drying the 8-hydroxyquinolinate salt in an oven at 80 °C for 24 hours (Table I).

A study of the effect of time on the reaction showed that at 55 to 60 °C the optimum reaction time for maximum sensitivity was 15 to 20 minutes (Table II). It was also observed that

TABLE VII

CONCENTRATIONS OF ALCOHOLS PRESENT IN THEIR PHTHALATE ESTERS

		Alcohol found, per cent. *m/m*		
			Vanadium 8-hydroxyquinolinate method	
Ester	Alcohol added, per cent. *m/m*	3,5-Dinitrobenzoyl chloride method	Toluene solution	Benzene solution
Di(2-ethylhexyl) phthalate	0·13	0·14, 0·13	0·14, 0·11	0·12, 0·14
Di(2-ethylhexyl) phthalate	0·33	0·34, 0·34	0·32, 0·29	0·34, 0·33
Diisooctyl phthalate	0·13	0·15, 0·14, 0·14, 0·15	0·14, 0·15	0·13, 0·14
Diisooctyl phthalate	0·35	0·40, 0·39, 0·36, 0·37	0·41, 0·40	0·41, 0·39
Di-Alphyl phthalate*	0·13	0·14, 0·14	0·14, 0·15	0·16, 0·14
Di-Alphyl phthalate*	0·33	0·33, 0·37	0·34, 0·34	0·33, 0·33
Dinonyl phthalate	0·14	0·16, 0·16, 0·18	0·14, 0·15	0·16, 0·15
Dinonyl phthalate	0·37	0·36, 0·38, 0·38	0·36, 0·37	0·37, 0·38
Diisooctyl phthalate (A)	0	0·011, 0·010, 0·009	0·009, 0·011, 0·010, 0·012	0·010, 0·013, 0·009, 0·009
	0·015	0·030, 0·028, 0·030	0·029, 0·026, 0·024, 0·027	0·029, 0·026, 0·025, 0·023
Diisooctyl phthalate (B)	0	0·016, 0·019, 0·019	0·017, 0·018, 0·019, 0·015	0·014, 0·016, 0·014, 0·018
	0·011	0·027, 0·032, 0·029	0·031, 0·028, 0·031, 0·029	0·027, 0·028, 0·030, 0·026
Diisooctyl phthalate (C)	0	0·033, 0·034, 0·036	0·036, 0·037, 0·035, 0·033	0·034, 0·036, 0·030, 0·033
	0·018	0·047, 0·048, 0·053	0·052, 0·052, 0·055, 0·050	0·054, 0·056, 0·044, 0·050

* Alphyl = ester of Alphanol (ICI registered trade-name) (C_7 to C_9 alcohols).

the blue-coloured complex formed on the addition of dichloroacetic acid deteriorated with time and therefore its absorbance should be measured within 15 minutes of the addition of acid (Table III).

The stability of the vanadium 8-hydroxyquinolinate reagent was also examined (Table IV). It deteriorated with time and therefore should be used within 24 hours of preparation.

In the light of these factors, an analytical procedure aimed at maximising the sensitivity and repeatability was devised. This procedure included the use of freshly prepared reagent, an internal standard and carefully timed stages.

3,5-Dinitrobenzoyl chloride method—

The 3,5-dinitrobenzoyl chloride method for determining small amounts of alcohol is based on the work of Johnson and Critchfield.[19] The alcohol reacts with 3,5-dinitrobenzoyl chloride in pyridine for 15 minutes. The 3,5-dinitrobenzoate formed is extracted and, on reaction with 1,2-diaminopropane, gives a red colour. When this procedure was applied to phthalate esters, it was found that the quality of the pyridine was critical (Table V) and its water content had to be reduced to less than 0·05 per cent. *m/V* in order to obtain the maximum red colour. It was also found that, as the intensity of the red colour decreased with time (Table VI), its absorbance had to be measured within 5 to 10 minutes of formation.

As with the 8-hydroxyquinolinate method, these limitations were minimised in the analytical procedure by adding an internal standard with a 10-μl Hamilton syringe.

TABLE VIII

COMPARATIVE RESULTS OF FREE ALCOHOL CONTENTS ON COMMERCIAL SAMPLES OF ESTERS USING THE TWO METHODS

		Free alcohol, per cent. *m/m*		
			Vanadium 8-hydroxyquinolinate method	
Ester	Sample No.	3,5-Dinitrobenzoyl chloride method	Benzene solution	Toluene solution
Di-(2-ethylhexyl) phthalate	1	0·03	0·02	0·03
	2	0·02	0·02	0·02
	3	0·10	0·09	0·11
	4	0·03	0·02	0·02
	5	0·02	0·02	0·02
Dinonyl phthalate	1	0·07	0·05	0·06
	2	0·12	0·13	0·12
	3	0·17	0·14	0·16
	4	0·03	0·02	0·01
Diisodecyl phthalate	1	0·42	0·40	0·40
	2	0·08	0·06	0·09
	3	0·18	0·20	0·18
	4	0·13	0·18	0·16
	5	0·11	0·12	0·11
	6	0·08	0·13	0·11
	7	0·22	0·23	0·20
Diisooctyl phthalate	1	0·09	0·10	0·09
	2	0·08	0·05	0·06
	3	0·02	0·02	0·03
	4	0·02	0·02	0·02
Di-Alphyl phthalate*	1	0·02	0·03	0·03
	2	0·04	0·03	0·03
	3	0·06	0·06	0·08
	4	0·02	0·02	0·02

* See footnote to Table VII.

Conclusions

Several phthalate esters were analysed for their C_8 to C_{10} alcohol contents using the standard procedures described, over the concentration range 0·01 to 0·4 per cent. *m/m*. These esters included some commercial samples and also phthalates that contained known added amounts of alcohol.

The results in Tables VII and VIII show that either reagent can be used to determine small amounts of alcohols. However, the 3,5-dinitrobenzoyl chloride method has two disadvantages: the test time is longer and the red colour obtained with 1,2-diaminopropane is unstable. The vanadium 8-hydroxyquinolinate method, in which either toluene or benzene is used as solvent, gives good repeatability but the sensitivity is lower when toluene is used and the sodium salt of vanadium 8-hydroxyquinolinate requires more careful preparation and drying.

Subsequent experience has confirmed the above conclusions, establishing that the vanadium 8-hydroxyquinolinate method is the preferred method. This method was also suitable for determining trace amounts of alcohols in adipates, glutarates, succinates and glycol ether acetates. It is not applicable to glycols, and phenolic antioxidants were found to interfere in both methods.

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Received *January 30th*, 1974
Accepted *April 8th*, 1974

Determination of Primary and Secondary Amines Alone and in Mixtures with Tertiary Amines

By BALBIR CHAND VERMA and SWATANTAR KUMAR

(*Department of Chemistry, Punjabi University, Patiala, India*)

An iodatometric method has been developed for the determination of primary and secondary amines that is based on their quantitative reaction with phenyl isothiocyanate in dimethylformamide to form substituted thioureas. These are titrated with potassium iodate in an acidic medium at room temperature. The end-point is detected visually by the yellow colour imparted to the solution by the first drop of the iodate solution in excess, and potentiometrically by using a bright platinum-wire indicator electrode and a saturated calomel reference electrode. Methods have also been developed for the determination of primary (or secondary) amines and tertiary amines in the presence of each other. An excess of phenyl isothiocyanate, added to the mixture in solution in dimethylformamide, converts the primary (or secondary) amines into the corresponding di- (or tri-) substituted thioureas, whereas the tertiary amines are left unreacted. The conductimetric titration of tertiary amines with trichloroacetic acid, followed by the iodatometric titration of thioureas formed, enables the mixture to be analysed for both components. The methods described are simple, accurate and reliable.

Primary and secondary amines react with organic isothiocyanates to yield substituted thioureas—

$$R'NH_2 + RNCS \longrightarrow \begin{matrix} RHN \\ \\ R'HN \end{matrix}\!\!>C = S$$

$$R'R''NH + RNCS \longrightarrow \begin{matrix} RHN \\ \\ R'R''N \end{matrix}\!\!>C = S$$

These reactions form one of the most general and convenient methods for the preparation of substituted thioureas and are widely used for the characterisation of amines and isothiocyanates. They can also be made the basis for the determination of an organic isothiocyanate or amine if one of them is allowed to react with a known excess amount of the other and, after completion of the reaction, the residual reactant is titrated with a suitable reagent. This method has been extensively used for the determination of isothiocyanates by allowing them to react with a known excess of amine and determining the unreacted excess of the amine acidimetrically. A similar method for the determination of amines by treating them with an excess of isothiocyanate has not, however, been applied, because of the non-availability of a suitable, direct method of titrating isothiocyanates.

Because the amine - isothiocyanate reaction to form thiourea proceeds to completion, the problem of the determination of isothiocyanates or amines can be made much simpler if a suitable method is made available for titrating the thiourea formed in the reaction. In the course of our extensive investigations on the oxidimetric determination of thioureas,[1] we have found that thioureas can be smoothly, rapidly and quantitatively titrated with potassium iodate[2] in an acidic medium at room temperature without a catalyst. No indicator need be added as the end-point is signalled by the appearance of the yellow colour due to iodine, resulting from the first drop of iodate solution in excess. The titrations can also be carried out potentiometrically by using bright platinum wire and a saturated calomel electrode assembly. The potentials attain stable values immediately on addition of each increment of the oxidant. A sharp jump in potential is observed at the equivalence point in each titration. In a recent publication,[3] we have reported a method for the determination of isothiocyanates that is based on their quantitative conversion into thioureas, which are then titrated with potassium iodate in an acidic medium.

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A simple and accurate method has now been developed for the determination of amines by their reaction with an excess of isothiocyanate and measuring the thiourea formed, again iodatometrically. The proposed method possesses some significant advantages over the acidimetric method that is in general use. Firstly, because of the oxidimetric nature of the proposed method, the determination of amines can be achieved in the presence of basic compounds that might otherwise cause interference in their acidimetric determination. Secondly, as tertiary amines do not react with isothiocyanates, the method permits the determination of primary (or secondary) amines in the presence of tertiary amines. Thirdly, potassium iodate is an oxidimetric standard, therefore the necessity of standardising acidic solutions in the acidimetric method can be eliminated. Lastly, the visible end-point iodatometric titrations can be performed without an indicator as the end-point is marked by the yellow tint imparted to the solution by the first drop of iodate solution in excess.

The proposed method consists in treating the amine with an excess of phenyl isothiocyanate in dimethylformamide and titrating the substituted thiourea formed iodatometrically in a sulphuric acid medium at room temperature. It should be mentioned here that the excess of isothiocyanate does not interfere in the iodatometric determination of thioureas.

Analysis of mixtures of primary (or secondary) amines and tertiary amines—

As tertiary amines are frequently encountered in the preparation of primary or secondary amines, the determination of primary (or secondary) amines and tertiary amines in the presence of each other is of great interest.

A simple and accurate procedure for the analysis of such a mixture in the same aliquot has been developed. The method consists in treating the solution of the mixture in dimethylformamide with an excess of phenyl isothiocyanate, when primary and secondary amines are converted into the corresponding di- and tri-substituted thioureas, respectively, and the tertiary amines are left unreacted. The tertiary amines are titrated conductimetrically with trichloroacetic acid followed by iodatometric titration of the substituted thioureas formed. The conductimetric titration corresponds to the amount of tertiary amine present, whereas the iodatometric titration gives the amount of primary (or secondary) amine in the mixture.

Experimental

Apparatus—

The redox potentiometric titrations were carried out with an Osaw Crompton (India) potentiometer incorporating an Osaw spot reflecting galvanometer, a bright platinum-wire indicator electrode and a saturated calomel reference electrode.

Acid - base conductimetric titrations were carried out with a Philips PR9500 conductivity bridge that operated at a frequency of 50 Hz. The cell electrodes consisted of two rigidly held square plates (approximately 0·8 cm^2 in area) of platinised platinum, facing each other at a distance of 1 cm.

A microburette of 10-ml capacity, graduated in 0·01-ml divisions, was used.

Reagents—

Dimethylformamide, commercial grade—This solvent was purified by standing it over AnalaR anhydrous sodium carbonate for 2 days, then decanting, distilling and fractionally distilling it, collecting the fraction distilling at 148·5 to 149·5 °C in an amber-glass bottle.

Potassium iodate solution, 0·05 N—This was prepared by dissolving 1·7834 g of the dried AnalaR grade solid in water and making the volume up to 1 litre.

Trichloroacetic acid solution, 1·0 N—This was prepared by dissolving slightly more than the calculated amount of the acid in water. The solution was then standardised by conductimetric titration[4] against anhydrous sodium acetate.

Phenyl isothiocyanate, Fluka, commercial grade—This material was distilled before use.

Ethylamine (50 *per cent. solution*) *and isopropylamine* (70 *per cent. solution*)—These were used as received from Riedel de Hähn.

n-Propylamine, n-butylamine, isobutylamine, diethylamine, triethylamine, pyridine, piperidine, pyrrolidine, α-picoline and quinoline, all commercial grade, were distilled before use.

All other chemicals used in this investigation were of analytical-reagent quality.

DETERMINATION OF PRIMARY OR SECONDARY AMINES—

Aliquots of solutions of each amine in dimethylformamide were taken in glass-stoppered titration flasks and 3 to 5 ml of phenyl isothiocyanate (an approximately 0·3 N solution in dimethylformamide) were added to each. The volume of the solution was made up to 10 ml with the solvent. The flask was then stoppered, swirled to mix the reactants, and set aside for 10 minutes to ensure completion of the reaction. Sufficient water and sulphuric acid to keep the normality of the solution at 2·0 to 2·5 in sulphuric acid and its volume at 100 ml were added. Each solution was cooled to room temperature (25 °C) and titrated with 0·05 N potassium iodate solution to the appearance of a distinct, permanent yellow colour.

If it is preferred, amylose (0·2 ml of a 1 per cent. aqueous solution) can be used as an ndicator, the solution acquiring a blue colour at the end-point.

In potentiometric titrations, the solution was magnetically stirred during the titration. A sharp jump in potential was observed at the equivalence point in each titration.

From the volume of standard potassium iodate (0·05 N) required to attain the end-point in visual and potentiometric titrations, the amount of substituted thiourea formed, and consequently the amount of amine, was calculated. The results are recorded in Table I.

TABLE I

IODATOMETRIC DETERMINATION OF PRIMARY AND SECONDARY AMINES

Compound	Amount found*/mg		Amount found†/mg	
	Visual method‡	Potentiometric method‡	Visual method‡	Potentiometric method‡
$CH_3CH_2NH_2$	9·94 ± 0·072	9·98 ± 0·036	39·85 ± 0·082	39·88 ± 0·056
$CH_3CH_2CH_2NH_2$	10·04 ± 0·034	10·03 ± 0·028	40·18 ± 0·063	40·16 ± 0·046
$(CH_3)_2CHNH_2$	9·97 ± 0·053	10·02 ± 0·051	40·28 ± 0·061	40·20 ± 0·025
$CH_3CH_2CH_2CH_2NH_2$ ..	10·01 ± 0·056	9·99 ± 0·041	39·72 ± 0·092	39·81 ± 0·057
$(CH_3)_2CHCH_2NH_2$ ..	9·96 ± 0·068	10·02 ± 0·038	40·28 ± 0·061	40·24 ± 0·047
$(CH_3CH_2)_2NH$	9·95 ± 0·076	9·95 ± 0·062	40·10 ± 0·075	40·02 ± 0·039
$CH_2CH_2CH_2CH_2NH$ ..	9·97 ± 0·063	9·98 ± 0·058	40·26 ± 0·056	40·21 ± 0·026
$CH_2CH_2CH_2CH_2CH_2NH$ ..	10·06 ± 0·075	10·05 ± 0·046	39·80 ± 0·078	39·82 ± 0·030

* Amount taken 10 mg.
† Amount taken 40 mg.
‡ Mean of six determinations, ± standard deviation.

DETERMINATION OF PRIMARY (OR SECONDARY) AMINES AND TERTIARY AMINES IN THE PRESENCE OF EACH OTHER—

Aliquots of solutions (in dimethylformamide) of synthetic mixtures with different ratios of primary (or secondary) amine to tertiary amine were taken in glass-stoppered titration flasks containing an excess (7 to 10 ml of an approximately 0·3 N solution) of phenyl isothiocyanate in dimethylformamide solution. The volume of each solution was made up to 20 ml with the solvent. Each flask was stoppered, swirled to mix the reactants and set aside for 10 minutes to ensure completion of the reaction. The solution was then mixed with 40 to 45 ml of approximately 1·0 N acetic acid, cooled to room temperature (25 °C) and titrated conductimetrically with standard 1·0 N trichloroacetic acid solution. To the same solution, sufficient water and sulphuric acid to keep the normality of the solution at 2·0 to 2·5 in sulphuric acid and its volume at 125 ml were added. The solution was cooled to room temperature and titrated potentiometrically with standard 0·05 N potassium iodate. The solution was stirred magnetically during potentiometric titrations.

The volume of standardised acid used in the acidimetric titration corresponds to the amount of the tertiary amine, whereas the volume used in the iodatometric titration gives the amount of di- (or tri-) substituted thiourea and consequently the amount of primary (or secondary) amine present in the sample. The results of the analysis of various ethylamine - triethylamine and diethylamine - triethylamine mixtures are recorded in Tables II and III, respectively.

Results and discussion

The results recorded in Table I show that ethylamine, n-propylamine, isopropylamine, n-butylamine, isobutylamine, diethylamine, piperidine and pyrrolidine can be determined visually and potentiometrically after conversion to the corresponding substituted thioureas with phenyl isothiocyanate in a dimethylformamide medium. The over-all standard deviations from the pooled results of all the visible end-point and potentiometric titrations performed with 10 mg of each amine have been found to be 0·062 and 0·045, respectively. For 40 mg of each amine, these values are 0·071 and 0·041, respectively.

Table II

Analysis of mixtures of ethylamine and triethylamine

Amount of $C_2H_5NH_2$ in the mixture/mg	Amount found*/mg	Amount of $(C_2H_5)_3N$ in the mixture/mg	Amount found*/mg	Ratio of $C_2H_5NH_2$ to $(C_2H_5)_3N$
20·00	20·12 ± 0·084	20·00	20·08 ± 0·126	1:1
20·00	20·14 ± 0·094	40·00	39·90 ± 0·132	1:2
20·00	19·90 ± 0·097	60·00	60·32 ± 0·094	1:3
20·00	20·06 ± 0·078	80·00	79·60 ± 0·127	1:4
40·00	39·85 ± 0·076	20·00	19·92 ± 0·088	2:1
60·00	59·72 ± 0·082	20·00	20·05 ± 0·095	3:1
80·00	80·36 ± 0·088	20·00	20·16 ± 0·096	4:1

* Mean of six determinations, ± standard deviation.

Amides (acetamide, urea, salicylamide and nicotinamide), imides (phthalimide and succinimide), Schiff's bases (*N*-*p*-chlorobenzylideneaniline and *N*-cinnamylideneanisidine) and tertiary amines (triethylamine, pyridine, α-picoline, quinoline and isoquinoline) do not cause any interference even when present in up to a five-fold excess in the determination of primary or secondary amines by the proposed method. Thiourea, thiosemicarbazide, thioacetamide, phenylhydrazine, xanthates, dithiocarbamates and organic isocyanates, however, do interfere. Thiourea, thiosemicarbazide, thioacetamide, phenylhydrazine, xanthates and dithiocarbamates interfere as they are oxidised by potassium iodate under the experimental conditions described for the determination of amines by the proposed method. If any of these compounds is therefore present in admixture with the listed amines, more potassium iodate than is required to oxidise quantitatively the substituted thiourea formed from amines (with phenyl isothiocyanate) will be consumed, and hence erratic results will be obtained. The interference caused by organic isocyanates is, however, attributed to their reaction with amines to form substituted ureas. The method could not be extended to the determination of aromatic amines as their reaction with the isothiocyanate was extremely slow.

Table III

Analysis of mixtures of diethylamine and triethylamine

Amount of $(C_2H_5)_2NH$ in the mixture/mg	Amount found*/mg	Amount of $(C_2H_5)_3N$ in the mixture/mg	Amount found*/mg	Ratio of $(C_2H_5)_2NH$ to $(C_2H_5)_3N$
20·00	19·92 ± 0·083	20·00	20·10 ± 0·124	1:1
20·00	20·10 ± 0·081	40·00	40·14 ± 0·156	1:2
20·00	19·88 ± 0·096	60·00	59·58 ± 0·141	1:3
20·00	19·96 ± 0·055	80·00	80·56 ± 0·104	1:4
40·00	40·22 ± 0·087	20·00	19·86 ± 0·088	2:1
60·00	60·35 ± 0·073	20·00	19·90 ± 0·114	3:1
80·00	79·55 ± 0·082	20·00	20·08 ± 0·096	4:1

* Mean of six determinations, ± standard deviation.

Amylose was not suitable in the visible end-point iodatometric determination of piperidine and pyrrolidine. The method proposed for the determination of amines has been called an iodatometric method because potassium iodate is used as an oxidimetric reagent to titrate the substituted thioureas formed from amines by reaction with phenyl isothiocyanate. The end-point of each titration is marked by the first distinct and permanent appearance of iodine

from the first drop of iodate solution in excess. Visually, the end-point can be detected either by the yellow colour that iodine imparts to the solution, or by the well known starch - iodide blue colour if amylose (a component of starch) is used as an indicator. The detection of the end-point by observing the yellow colour due to iodine is found to be satisfactory in the titrations of each of the listed amines by the above method. However, when amylose is used as the indicator, the change from colourless to blue is sharp in titrations of all of the listed amines except piperidine and pyrrolidine. Thus, amylose is not a suitable indicator for titrations of thioureas formed from piperidine and pyrrolidine, by reaction with phenyl isothiocyanate, with potassium iodate in an acidic medium.

The proposed methods for the determination of primary (or secondary) amines and tertiary amines in the presence of each other, besides being simple, accurate and reliable, have the added advantage that the analysis can be conducted on the same sample solution, thus saving time and effort. The changes in slope produced at the equivalence points in the conductimetric titrations of tertiary amines are sharp enough to allow easy location of the end-point, thus giving accurate results. Synthetic mixtures of ethylamine and triethylamine with the ratios in the range from 1 : 4 to 4 : 1 can be analysed with an average standard deviation of 0·31 per cent., in the instance of ethylamine, and 0·35 per cent., in the instance of triethylamine (Table II). The replicate samples of any amine under investigation were not weighed individually, but rather a single, large sample was weighed, dissolved in a known volume of dimethylformamide and aliquots taken for analysis in the titration vessels. That the replicate aliquots of an amine solution delivered the same amount of amine each time was checked by independent acidimetric titrations of that amine in several such aliquots. This check was applied to all the amines, including ethylamine and isopropylamine, which are available as 50 and 70 per cent. aqueous solutions, respectively. The method has also been applied to mixtures of pyridine, α-picoline, quinoline or triethylamine with n-propylamine, n-butylamine and isobutylamine. The results agree well with those of the tabulated mixture

The results recorded in Table III for the analysis of synthetic mixtures of diethylamine and triethylamine, also in ratios from 1:4 to 4:1, show that the mixture can be analysed with an average standard deviation of 0·29 per cent. (for diethylamine) and 0·41 per cent. (for triethylamine), respectively. The method has also been extended to other mixtures, such as diethylamine with pyridine, α-picoline or quinoline and piperidine and pyrrolidine with pyridine, α-picoline, quinoline or triethylamine, which have been determined to the same accuracy.

The authors thank the Council of Scientific and Industrial Research (India) for the award of a fellowship to one of them (S.K.).

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Received *December 19th*, 1973
Accepted *February 8th*, 1974

Analyst, August, 1974, Vol. 99, *pp.* 503–514

A Technique for the Determination of Trace Anions by the Combination of a Potentiometric Sensor and Liquid Chromatography, with Particular Reference to the Determination of Halides*

By M. C. FRANKS and D. L. PULLEN

(*BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex, TW16 7LN*)

A technique for the determination of trace amounts of halides in the presence of other ions is described. The species are separated by means of liquid chromatography and detected potentiometrically by a silver - silver chloride micro-electrode. The technique readily lends itself to automation and an apparatus for rapid, repetitive analyses has been designed. By careful choice of the eluting agent and stationary phase it is possible to achieve a variety of separations, *e.g.*, the determination of chloride in the presence of excess of sulphide, the separation and determination of nanogram amounts of chloride, bromide and iodide in mixed halide solutions and the determination of chloride in boiler waters.

In addition, conditions for the extension of the technique to the separation and determination of other anions are proposed.

Although there are many methods available for the determination of trace amounts of metals, including highly developed polarographic procedures and a variety of spectroscopic methods, there are few techniques that are of general application to the detection and determination of trace amounts of anions. The basis of the technique described in this paper is the separation of anions by liquid chromatography and their determination potentiometrically by an ion-selective micro-electrode, a combination that ensures high degrees of specificity and sensitivity.

Until fairly recently, the advantages of an electrode as a potentiometric detector in a flowing solution have not been fully appreciated. In reviews of liquid-chromatographic detectors,[1,2] potentiometry is given only a brief mention. This is surprising as potentiometric detectors are used throughout industry for the control of pH and sodium[3] and ammonia concentrations.[4] For the determination of anions, modern ion-selective electrodes have proved to be successful sensors for fluoride,[5] sulphide,[6] cyanide[7] and chloride[8] and similar flow-through systems have also been described in biomedical research. However, in these instances no chromatography was involved. In this work, the importance of the chromatographic process is emphasised as it can both replace difficult separations and enable non-selective sensors to be used as specific detectors. A particular aspect of the potentiometric detector has also been given special attention, namely its use at very low concentration levels, near to the limit of detection. It is in this region that the detector is useful for trace analysis.

In order to illustrate the technique the procedure for a specific application, trace halide analysis, is described in detail in the experimental section of this paper. It includes an account of the use of a rapid, repetitive automatic analyser for the continuous measurement of chloride samples in the parts per million concentration range in microlitre volumes. The theoretical aspects of electrode response have received comprehensive treatment elsewhere[9] and are considered only briefly here. The potentialities of the technique for general application to the determination of anions are also considered, exploratory work is described and the implications are discussed.

Experimental

Description of the apparatus—

A block diagram is shown in Fig. 1. A micro-scale metering pump circulates the mobile phase through a narrow-bore plastic tube containing the stationary phase. At the end of the tube is a micro-electrochemical detector, which is connected through a high-stability amplifier to a chart recorder. A few microlitres of sample solution are injected into the stationary phase, either manually through a septum, or automatically through a sampling valve. The

* Presented in part at the Third SAC Conference, Durham, July 12th to 16th, 1971.

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mobile phase carries the sample to the detector where the species to be determined is detected and registered as a peak on the recorder chart.

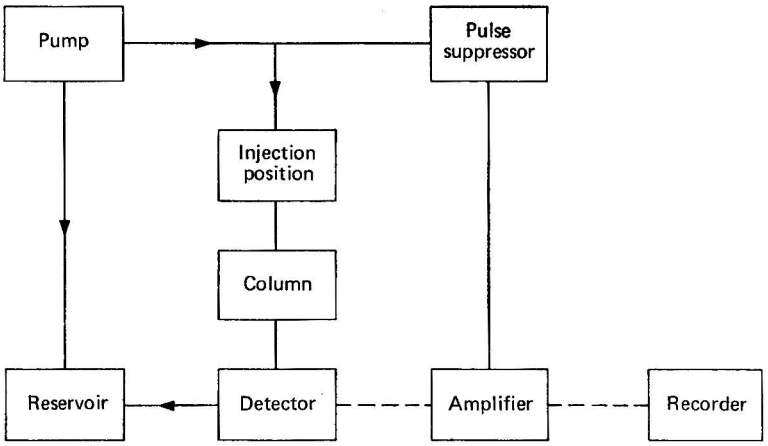

Fig. 1. Block diagram of the apparatus

The choice of the detector and the nature of the stationary and mobile phases depend on the anion to be determined and the known interferences from other constituents of the sample. Our aim was to determine trace amounts of halides in solution and our attention was mainly directed towards ion-exchange processes. Accordingly, in the description of the major parts of the apparatus, the detector is illustrated by the silver - silver chloride electrode and the chromatographic column is described with reference to ion-exchange resins.

The major components of the equipment are as follows.

Pump—A micro-scale metering pump capable of delivery against a pressure resistance of 10 bar at a flow-rate of 0·2 to 0·6 ml min^{-1}. The pump used was made by F. A. Hughes (Epsom, Surrey) and had a stainless steel piston with a reciprocating frequency of 20 strokes per minute.

Pulse suppressor—An effective antipulsation device, which reduces electrode potential oscillations caused by the pulsing characteristics of the pump, consists of a coil of polythene tubing 50 cm long and of 3·2 mm i.d., which is full of air.

Reservoir—A 2-litre polythene bottle is convenient for holding the mobile phase.

Flow meter—A Meterate flow meter tube, manufactured by Glass Precision Engineering (Hemel Hempstead, Herts.) Limited, was used (meter size A); glass spherical float range, 0 to 0·5 ml min^{-1}.

Sample injection—For manual operation the injection point comprises a T-piece containing a silicone rubber septum, held between metal washers, with a small central hole large enough to take a micro-syringe needle. A Hamilton micro-syringe proved to be satisfactory. A needle guide was found to be useful for ensuring correct insertion of the needle.

For automatic operation, *i.e.*, for monitoring a plant stream, a conventional sliding valve injection device is situated at the top of the chromatographic column and the sample is injected directly on top of the ion-exchange resin.

Chromatographic column—This consists of a length of polythene or nylon tubing packed with the appropriate stationary phase. For low-pressure chromatography, *i.e.*, less than 7 bar, 1·6 or 3·2 mm i.d. polythene tubing is suitable, whereas for higher pressure work, nylon tubing of similar dimensions is required. In the determination of chloride, the stationary phase was an ion-exchange resin in the metal form. The resin, initially in the sodium or hydrogen form, was converted by three treatments with excess of a metal salt, preferably the sulphate, acetate or nitrate. The resin in the metal form was then washed by decantation at least ten times with distilled water in order to remove fines and excess of metal salt. One end of the tubing (approximately 160 cm) was heated gently and moulded into a narrow tip (the bottom of the column) into which a small cotton-wool plug was placed. The column was packed in one rapid process by applying suction from the bottom of the column while the top

was held in a suspension of the stationary phase, *e.g.*, ion exchanger in distilled water, 1 + 1 by volume. The column was connected to the micro-pump and a solution of eluting agent was pumped at a flow-rate of 0·3 to 0·4 ml min^{-1} for several hours in order to settle the resin, after which the column was cut to the desired length and connected to the injection valve.

Detector system—The detector system consists of two silver - silver chloride electrodes; one is used as a reference while the other is placed in the flowing stream. A detailed diagram of the detector is shown in Fig. 2. The design of the detector is such that it is easy to replace electrodes in the housing, and the dead volume around the indicator electrode is kept to a minimum (50 μl or less). The housing and electrode barrels are made from Pyrex glass, the latter being ground down just sufficiently to allow a steady flow of circulating eluting agent through the housing. Quickfit and Quartz screw-thread adaptors are used as connectors. A 3-mm length of 3·2 mm diameter silicon carbide rod, grit type F, purchased from AEI, Manchester, was sealed into the glass wall to provide a restrained flow liquid junction between the reference and indicator electrodes.

Electrodes—These were made from 16 s.w.g. silver wire, shaped as in Fig. 2, and chloridised in hydrochloric acid. Conditions were controlled to produce batches of electrodes with bias potentials of less than 0·2 mV.

Instrumentation—The two essential units are a buffer amplifier and a recorder. In some of the preliminary work a Keithley or Vibron Model 33B-2 electrometer was used to measure the potential changes and to investigate electrical parameters. The present apparatus has a purpose-built amplifier, the chief characteristics of which are low bias current, low noise and high impedance. Incorporated into the amplifier are a damping device and a narrow-band filter to remove residual oscillations of the output potential.

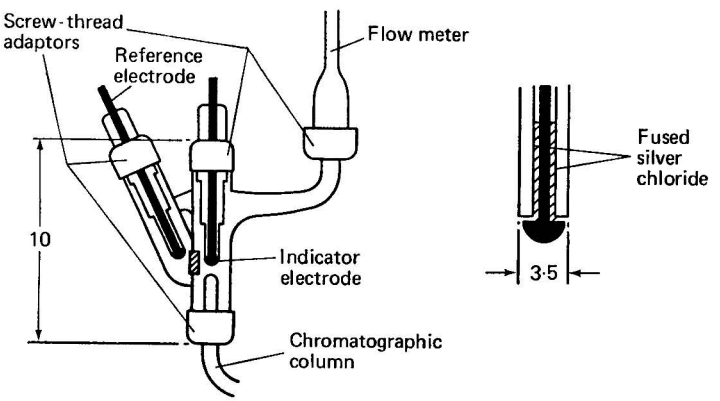

Fig. 2. Assembled detector (dimensions are given in millimetres)

The signal can be displayed on any paper chart recorder that matches the output characteristics of the amplifier; a floating input will be necessary. A Vitatron UR 400M recorder, supplied by Fisons Scientific Apparatus Limited, is the least expensive that has been used satisfactorily.

Careful attention to the screening of connecting leads is necessary in order to avoid excessive interference noise on the recorder trace. Co-axial cable is used for both input connections to the amplifier; screened leads are satisfactory elsewhere. The high-impedance terminal of the amplifier should always be connected to the reference electrode, regardless of polarity, and this lead should be kept as short as possible. From the indicator electrode there are resistive and capacitative paths to earth through the circulating liquid, which is in contact with the earthed frame of the pump. It is therefore necessary to connect all screening to this point. The screening of the leads to the recorder should not be connected to the earthed recorder frame. The whole apparatus is enclosed in a metal box that acts as a Faraday cage to screen the detector from external electrical interference.

ASSEMBLY OF THE APPARATUS—

The apparatus is assembled in the following sequence to avoid inclusion of air: first, the reservoir, pump and pulse suppressor; then, with the pump running, the injection valve,

chromatographic column, detector and flow meter are connected. Plastic tubing and 6·35 mm i.d. Simplifix brass connectors are used to link the components. When the mobile phase has passed through the sintered grit into the reference electrode compartment the latter is topped up with the mobile phase and the electrode inserted. The electrical connections are made, the pump is set to the required flow-rate and the apparatus left running for several hours in order to stabilise it.

Operation of the apparatus—

A small volume of sample is injected into the column. If the operation is carried out manually, the sample is added with a micro-syringe, making sure that the tip of the needle passes through the centre of the T-junction and into the ion-exchange resin. The sample is carried by the mobile phase to the detector, where the chloride is detected and shown on the recorder as a chromatographic peak. The sample volume is chosen so that the peak height is 5 mV or less as it is in that region that the peak height is proportional to the chloride content. A maximum volume of 100 μl is observed in order to prevent peak broadening.

Reagents—

Water—Water with a very low chloride content, preferably below 20 p.p.b. (parts per 10^9), is used to prepare all of the standard solutions and mobile phases and to wash all the apparatus. Suitable water can be produced by de-ionisation of laboratory distilled water so as to obtain a specific resistance of greater than 4 MΩ cm. The chloride content can be determined, when necessary, by the method of Rodabaugh and Upperman.[10] Polythene bottles with screw-caps are convenient for storing the de-ionised water.

Standard chloride solution A—Dry analytical-reagent grade sodium chloride in an oven at 250 to 350 °C for 1 to 2 hours, then weigh 1·649 g of it into a 1-litre Pyrex glass calibrated flask, dissolve it in water and dilute to the mark with water. This solution should be stable for at least 1 year.

1 ml of solution A ≡ 1000 μg of chloride.

Standard chloride solution B—Transfer 10 ml of the standard solution A with a pipette into a 500-ml Pyrex calibrated flask, dilute to the mark with water and mix. We found this solution to be stable for at least 6 months.

1 ml of solution B ≡ 20 μg of chloride.

Other halide solutions—Prepare 1000 and 20 p.p.m. solutions of bromide and iodide by weighing and diluting appropriate amounts of the sodium salts of these halides as described above. Prepare the iodide solutions just before use.

Ion-exchange resins—Strongly acidic resins, Chromatographic grade, 200 mesh, were obtained from Permutit Company Limited.

All other chemicals used were of analytical-reagent grade unless otherwise specified.

Temperature—

The room temperature varied between 19 and 21 °C.

Evaluation of the detector—

There are few useful references to the behaviour of a potentiometric detector in a flowing solution of low ionic activity, especially at the low detection limit set by solubility considerations. A major part of the initial work was to establish that stable electrode potentials could be obtained under such conditions. This attempt involved the design of a micro-cell and associated instrumentation that would produce suitably low noise signals. Some aspects of this work and a simple theoretical treatment are described.

Theory of the response of the silver chloride electrode—

The potential of the silver - silver chloride system is given by

$$E = E^\circ_{Ag/Ag^+} + \frac{RT}{F} \ln K_s - \frac{RT}{F} \ln \gamma M_{Cl^-} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where E°_{Ag/Ag^+} is the standard electrode potential, R is the gas constant, T is the absolute

temperature, F is the Faraday constant, M_{Cl^-} is the chloride-ion concentration and γ is the activity coefficient of the chloride ion. The change in potential, ΔE, in a constant ionic medium, when the chloride concentration changes to M'_{Cl^-}, is given by

$$\Delta E = \frac{RT}{F} \ln\left(\frac{M'_{Cl^-}}{M_{Cl^-}}\right) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

When the silver chloride electrode is immersed in a solution a certain amount of silver chloride dissolves from the surface. If the chloride concentration of the solution before immersion is x, then at equilibrium the total concentration of the chloride ions, M'_{Cl^-}, is given by

$$M'_{Cl^-} = \frac{x}{2} + \sqrt{\left(\frac{x}{2}\right)^2 + K_s} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where K_s is the solubility product of silver chloride. For a chloride-free solution, when $x = 0$, $M_{Cl^-} = \sqrt{K_s}$.

When a chloride-free solution is replaced by one containing chloride ions, we have, from equations (2) and (3)—

$$\Delta E = \frac{RT}{F} \ln\left[\frac{x}{2\sqrt{K_s}} + \sqrt{\left(\frac{x}{2\sqrt{K_s}}\right)^2 + 1}\right] \quad .. \quad .. \quad .. \quad (4)$$

When $\frac{x}{2}$ is small compared with $\sqrt{K_s}$, (4) becomes

$$\Delta E = \frac{RT}{F} \times \frac{x}{2\sqrt{K_s}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5)$$

For an electrode in a flowing solution at equilibrium, (5) can be written—

$$\Delta E = \frac{RT}{2F}\left(\frac{\text{added chloride concentration}}{\text{initial chloride concentration}}\right) \quad .. \quad .. \quad .. \quad (6)$$

Thus, the change in potential is proportional to the added chloride concentration (x). The sensitivity of the detector is obtained from the $\frac{RT}{2F\sqrt{K_s}}$ term in equation (5) and is 3·3 mV per 0·1 p.p.m. of chloride at 20 °C.

Bardin,[9] in a more detailed account, showed that this approximation held for concentration changes up to 0·2 p.p.m. of chloride. By analogy it can be shown that low concentrations of other anions with insoluble silver salts should produce potential changes proportional to the concentrations in which they were added.

Confirmation of theoretical response—It was shown experimentally that the electrode response was proportional to chloride concentrations of up to 0·2 p.p.m. and that the sensitivity was 3 mV per 0·1 p.p.m. of chloride. These findings were obtained with flowing solutions at 20 °C. The linearity of the calibration graphs from many other experiments, and those shown in this paper, also confirm the proportional response that was expected at low halide concentrations.

Experimental conditions that affect electrode response are discussed in the next three sections.

Effect of flow-rate—The potential of the silver chloride detector was measured under flowing and static conditions with chloride concentrations from 10^{-5} to 10^{-4} M. The flow-rates were in the range from 0·1 to 1·0 ml min^{-1}. As expected, the potential of the electrode was found to vary with the flow-rate. The change in potential from a static to a flowing condition could be as large as 30 mV and was found to depend on various parameters, such as the nature and concentration of the mobile phase, the nature of the stationary phase and the material of construction of the column, *i.e.*, polythene or nylon. It is not possible to state

unequivocably what underlying phenomena produced these potential changes. Although this aspect is of some theoretical interest, it was not investigated in detail as we were primarily concerned with the practical problems of measuring the potential variation, noise level and base-line change in a constant-flow system. Provided that the flow-rate is maintained within ± 2 per cent., the change in electrode potential is small and has little effect on the repeatability of results that are obtained.

Effect of temperature—The effects of temperature on the detector are complex. The potential of both the reference and detecting electrode will vary with the factor $\frac{RT}{F}$ and the solubility term ln K_s, which is itself temperature dependent. The practical effects of change in temperature are two-fold. A change in base-line occurs on the recorder trace and the sensitivity to chloride alters [as shown in equation (5)]. The effect of temperature on sensitivity was tested over the range from 17 to 23 °C by equilibrating the whole apparatus before measuring the electrode response. A decrease in sensitivity of 2 per cent. per 1 °C rise was found. This is in agreement with the theoretical expectations of equation (5) from consideration of the combined effect of the $\frac{RT}{F}$ term and the $\sqrt{K_s}$ term, the latter having the larger temperature coefficient. By controlling the temperature to ± 1 °C sufficient accuracy for calibration and measurement was obtained for our needs, and the normal base-line drift was small enough to enable small peaks to be easily discerned.

Effect of light—As silver chloride electrodes are known to be photosensitive the detector assembly was shielded from direct light.

Calibration—

Calibration graphs were obtained by injecting a series of known halide solutions that had been prepared by dilution of the standard halide solution. The peak height was plotted against halide content. The calibration graphs were linear (Figs. 3 and 4) except at high chloride concentrations where the Nernstian logarithmic relationship was found.

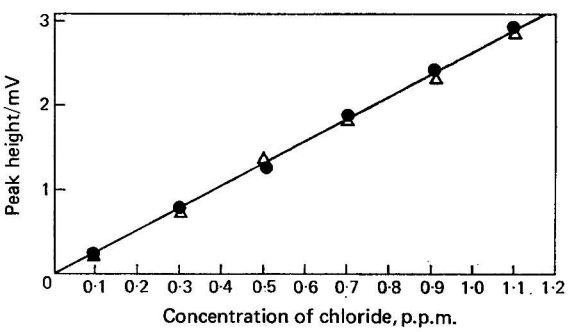

Fig. 3. Calibration graph for chloride in high-pressure boiler water: ●, chloride in pure water; and △, simulated boiler water (Table I) *plus* added chloride (sample volume 20 μl)

Stability of electrode response—

In the supporting work, in addition to confirming the theoretical response it was also established that the potential of an electrode in a high, but constant, velocity stream is surprisingly stable. In the apparatus described above the cell and instrumentation were carefully designed such that the normal base-line peak-to-peak noise is less than 15 μV. The daily base-line non-cumulative drift is less than 0·2 mV for most of the analytical applications.

Base-line stability in the order of less than 15 μV is essential if the technique is to be used for the determination of nanogram amounts of halide, because the concentration of a sample

is considerably reduced by the chromatographic separation. If it is assumed that the minimum detectable signal is twice the base-line noise level, then from Fig. 5, the limits of detection of chloride, bromide and iodide in this particular system are 4, 1 and about 2 ng, respectively. Similarly, the limit of detection for other systems will depend on the response and the noise level.

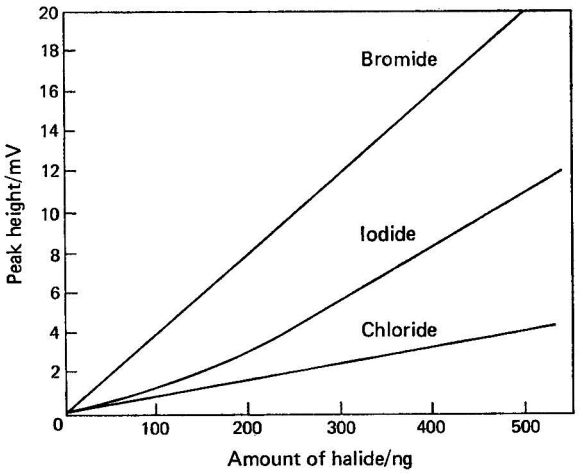

Fig. 4. Calibration graphs for chloride, bromide and iodide. Cadmium acetate chromatographic system

Concerning the lower limit of detection that it is possible to attain, we think that with careful electrical shielding and noise reduction, stability of the order of microvolts can be obtained in a flowing solution. We agree with Light[11] that "when this stability is achieved electrodes will obey the Nernst equation much better than is generally believed."

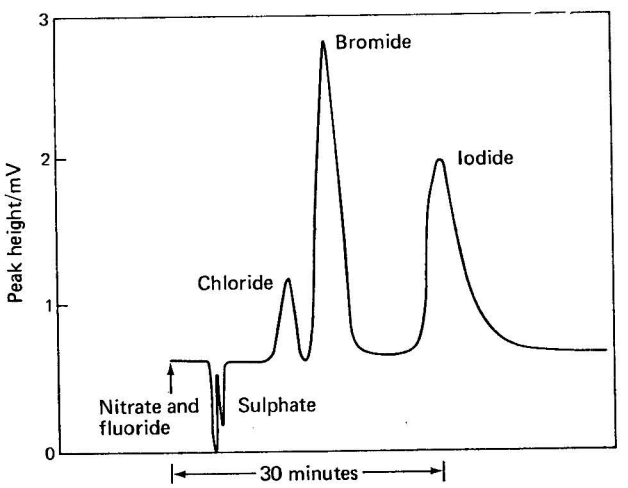

Fig. 5. Separation of chloride, bromide and iodide in presence of fluoride, sulphate and nitrate. Stationary phase, Zeo-Karb 225, cadmium form; mobile phase, 0·0025 M cadmium acetate, flow-rate 0·4 ml min^{-1}. Sample 50 μl, containing 1000 p.p.m. of sulphate, nitrate and fluoride with 1 p.p.m. each of the other halides

APPLICATIONS

A few of the applications of the technique to the determination of chloride are described below. In each instance the silver - silver chloride electrode is used. The applications have been selected to show how interferences can be removed by chromatographic separation or suppressed by use of a suitable mobile phase.

DETERMINATION OF CHLORIDE IN THE PRESENCE OF SULPHIDE—

Corrosion problems in crude oil distillation units have been the concern of the petroleum industry for many years and require the monitoring of chloride at the parts per million level in the aqueous overheads. Large excesses of sulphide and thiols are present together with some oxy-acids of sulphur. The chloride content of aqueous overheads can be determined by conventional potentiometric or visual indicator titrations, after removal of the interfering substances, but these methods are time consuming. It was found, after preliminary investigational work, that chloride could be separated completely and quantitatively from all interfering substances by use of a chromatographic column consisting of the lead form of a cation exchanger as the stationary phase and dilute lead acetate solution as the mobile phase.[12]

When a small sample, *e.g.*, 15 μl, is injected into the chromatograph, sulphide and thiols are precipitated as the insoluble lead salts, while the other sulphur compounds are separated from chloride ions by the ion-exchange system. Suitable chromatographic conditions were as follows: stationary phase, a nylon column 80 cm long, i.d. 1·6 mm, filled with Zeo-Karb 225 in the lead form, 200 mesh; mobile phase, a 0·001 M lead acetate solution, flow-rate 0·4 ml min^{-1}.

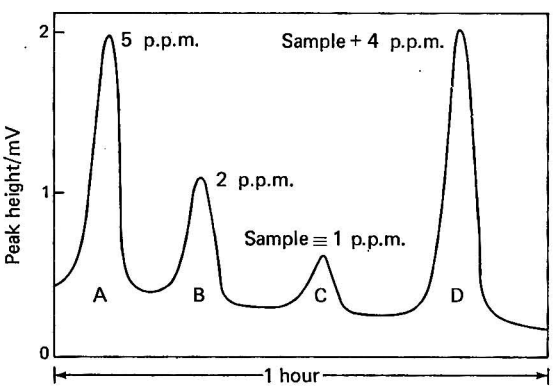

Fig. 6. Typical chloride peaks from automated laboratory analyser. A and B are calibration peaks; peak C is from a sample of aqueous overheads (S) containing 1 p.p.m. of chloride *plus* about 100 p.p.m. of sulphide; and peak D is sample (S) *plus* 4 p.p.m. of chloride. Sample volume, 15 μl; retention time, 6 minutes

A typical set of calibration and sample peaks is shown in Fig. 6. The recovery of chloride is greater than 95 per cent. and as little as 0·5 p.p.m. of chloride can be measured with an elapsed time of 15 minutes. The basic apparatus has been converted for use as an automatic laboratory analyser by injecting samples from a sliding valve device controlled by a simple programmer. This analyser was run successfully for several months to determine the chloride content of several hundred samples from aqueous overheads before a marked deterioration in apparatus response and a build-up of pressure necessitated renewal of the column and detector. A statistical analysis of fifty samples, tested in duplicate, gave a standard deviation of 0·3 p.p.m. of chloride for levels below 10 p.p.m. Twenty samples covering the range 0 to 100 p.p.m. were tested by use of the automatic analyser and by a standard chemical procedure. There was no significant bias.

CHLORIDE IN BOILER WATERS—

The determination of trace amounts of chloride in boiler water presents a somewhat similar problem to that described above. Although standard chemical procedures exist they still require considerable time and manipulation. The main types of boilers give rise to three kinds of boiler waters, which normally contain the substances listed in Table I, present either as additives or contaminants.

TABLE I

TYPICAL BOILER-WATER COMPOSITIONS

Boiler type	Sample constituents	Concentration, p.p.m.
High pressure (120 bar)	Chloride	0–1
	Silica	0–2
	Ammonia	0–5
Medium pressure (40 bar)	Chloride	0–10
	Phosphate	0–70
	Hydrazine	0·1–1
	Silica	0–25
Low pressure	Chloride	0–10
	Phosphate	0–50
	Sulphite	0–150
	Sulphate	0–300

The lead form ion exchanger and lead acetate eluting agent system described previously cannot be used as large amounts of chloride are lost, presumably by adsorption on to the lead phosphate precipitate. (We have found that since this work was carried out the co-precipitation of chloride on to lead phosphate has been put forward as the basis of a method for determining amounts of chloride of the order of parts per billion.[10]) After some initial experimentation the following conditions were found to be suitable for the determination of chloride in all types of boiler waters: stationary phase, a column 10 cm long and of 3·2 mm i.d., containing 200-mesh powdered polythene; mobile phase, 100 p.p.m. orthophosphoric acid solution, flow-rate 0·3 ml min^{-1}. The orthophosphoric acid has the effect of suppressing to a practically constant level the small but insignificant interference due to varying levels of phosphate in actual boiler waters. The powdered polythene placed between the injection points and the silver - silver chloride detector merely acts as a convenient means of presenting the sample to the detector so that a reproducible peak is obtained. No chromatographic separation is needed as constituents other than phosphate do not interfere significantly.

In Figs. 3 and 7 calibration graphs obtained by adding known amounts of chloride to simulated water from low and high-pressure boilers are shown. By use of these graphs we have analysed a large number of samples and found that for low-pressure boiler waters the

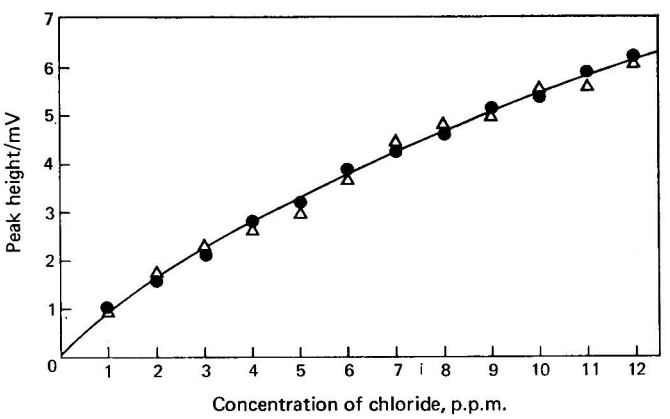

Fig. 7. Calibration graphs for chloride in low-pressure boiler water: ●, chloride added to distilled water; and △, chloride added to synthetic boiler water (Table I). Peak heights are corrected for a boiler water blank ≡ 0·9 mV to show coincidence of the graphs

results of determinations with this analyser and standard chemical analyses generally agree to within 0·1 p.p.m. of chloride. As an example of the performance that can be achieved at low chloride levels, high-pressure boiler waters containing 0 to 1 p.p.m. of chloride were analysed with a standard deviation of 0·02 p.p.m. (30 degrees of freedom) at a rate of 30 samples per hour.

DETERMINATION OF HALIDES IN MIXTURES—

The first two applications were concerned solely with the determination of chloride. It was observed in the supporting work for these applications that, with certain chromatographic systems, there was an indication of the separation of bromide and iodide.

A literature search revealed many ion-exchange systems that are capable of separating the halides in mixtures. However, most of these methods are lengthy, requiring 3 to 4 hours, apply to milligram or greater amounts, and make use of eluting agents that affect the response of the silver - silver chloride electrode. A thin-layer chromatographic system that involved the use of cadmium salts as eluting agents was found[13] that appeared to be suitable. After a preliminary study of the factors affecting the separation, namely the degree of cross-linking of the resin, the resin mesh size and the strength of the eluting agent, the following conditions were arrived at for complete separation of the halides: a column 80 cm long, 3·2 mm in i.d., packed with 4 to 5 per cent. cross-linked Zeo-Karb 225 (cadmium form), 200 mesh, as the stationary phase; and a 0·0025 M cadmium acetate solution, flow-rate 0·4 ml min^{-1}, as the mobile phase.

In Fig. 5 the chromatogram is shown that is obtained from the injection of a 50-μl sample of a solution containing 1 p.p.m. each of chloride, bromide and iodide in the presence of 1000 p.p.m. of some common anions. The halides are well resolved and are eluted in order of the increasing ionic strength of their cadmium complexes. Under the most suitable experimental conditions it is possible to separate and determine a minimum of 4 ng of each halide. This technique is therefore much more sensitive than existing electrochemical or spectrophotometric procedures.

Interferences in halide determination—It is possible to predict, in qualitative terms, the effect of various anions on the silver - silver chloride detector. As expected, sulphate, nitrate, perchlorate, fluoride and other anions with soluble silver salts do not interfere, either as salts or as free acids, even at 1000 times the concentration of the halide. They are also eluted before chloride. Other anions with insoluble silver salts, *e.g.*, chromate and thiocyanate, or which form silver complexes, *e.g.*, thiosulphate and cyanide, produce a response at the electrode the magnitude of which depends on the solubility product or the instability constant of the resulting compound.

In Table II, the relative elution times of some anions are given. Because of the low responses of certain anions or the wide separation of peaks (Table II), it can be seen that only sulphide, thiocyanate and thiosulphate can possibly interfere in the determination of halide to any extent. The degree of interference depends on the relative amounts of interferent and ion sought.

TABLE II

RELATIVE ELUTION TIMES OF SOME ANIONS WITH ZEO-KARB 225 (CADMIUM FORM) AS STATIONARY PHASE AND 0·0025 M CADMIUM ACETATE AS ELUTING AGENT

F^-, CrO_4^{2-}, NO_3^-	SO_4^{2-}, $H_2PO_4^-$	$S_2O_3^{2-}$, S^{2-}	Cl^-	Br^-	CNS^-	I^-	CN^-
0·5	0·6	0·8	1·0	1·25	2·0	2·2	10

FURTHER APPLICATIONS—

It is evident that the system described above has numerous practical applications in trace analysis. A few such applications which have been successfully carried out in our laboratory include: the determination of halides in well waters; the determination of chloride in the presence of a large excess of fluoride, phosphate and sulphate; the direct determination of bromide in sea water, in which the chloride to bromide ratio is about 3000:1 (Fig. 8); and the determination of chloride in concentrated cyanide solutions. The system has also been used to determine halogens in solutions resulting from Schöniger flask

and Wickbold apparatus combustions. In the latter procedure, high results or large blanks can be produced if the chloride is determined by the mercury(II) thiocyanate colorimetric technique,[14] or by titration with silver or mercury salts, especially if gram amounts of sample are burnt. It has been conclusively shown, by using the chromatographic - potentiometric technique, that these "high blanks" are due to reactions with the products of combustion and not to chloride. Finally, it should be emphasised that the applications mentioned above have often simplified analyses previously requiring time-consuming classical methods.

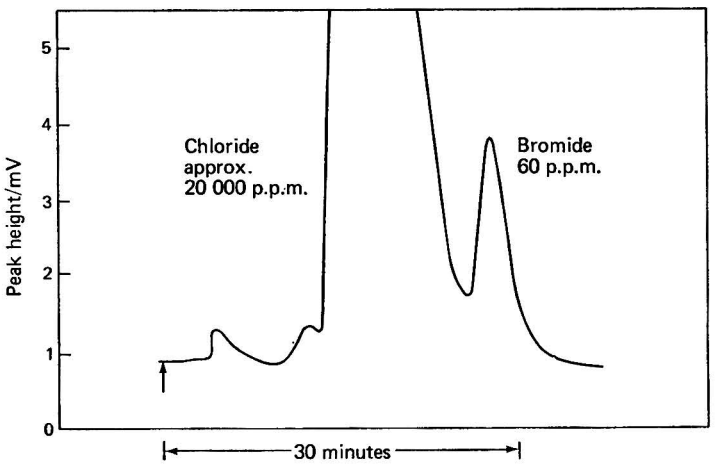

Fig. 8. Direct determination of bromide in sea water. Conditions: cadmium acetate chromatographic system, flow-rate 0·2 ml min^{-1}; sample size, 1 μl. The bromide content was found to be 60 p.p.m., compared with the chemically determined value of 65 p.p.m.

Discussion

Although other workers have proposed the use of ion-selective electrodes as liquid chromatographic potentiometric detectors,[15] we have not been able to find examples of the practical application of this technique in the literature. Our work has shown that not only is this technique suitable for the determination of the concentration of anions in flowing solutions, but that it can be used for their quantitative measurement at the parts per million level and it is also capable of determining halides in mixtures after a single chromatographic separation. A limiting factor in the separation and determination of complex mixtures is the possibility of irreversible changes taking place at the electrode surface.

Some aspects of this limitation were observed in the initial calibration runs with mixed halide solutions. At first the response to chloride decreased rather rapidly and the base-line level changed between the halide peaks. However, if the electrode is conditioned by circulating the mobile phase, containing a few milligrams of mixed halides, overnight, the response becomes practically constant from then onwards and the base-line becomes horizontal (see Fig. 5), with the result that the analysis of mixed halide solutions becomes easier and more precise. The sensitivity to bromide and iodide is essentially that expected from the theoretical electrode response and chromatographic dilution, but the sensitivity to chloride is much lower, presumably because of the formation of a mixed halide electrode (Fig. 4).

The success of the technique for the determination of amounts of halides of the order of nanograms suggested that this special application could be extended to the general determination of other anions, and further work has indicated that there is every possibility of doing this.

By using the cadmium acetate chromatographic system and a silver - silver chloride electrode we have been able to determine thiosulphate and cyanide in concentrations in the parts per million region.

The Orion liquid-state ion-selective electrode, which has been recommended for the determination of the chloride ion but which is also sensitive to other anions, has been produced in miniature. With this micro-electrode as a detector and the same chromatographic system

as above we have been able to detect amounts of nitrate and perchlorate in the parts per million range.

The combination of a lead amalgam electrode and cellulose-column chromatography has permitted the separation and determination of sulphate and phosphate ions. By means of a barium sulphate impregnated silicone-rubber electrode, trace amounts of sulphate and phosphate in solution have been determined. Further work is required in order to extend the life of the electrode and increase the sensitivity of these procedures and it is hoped to publish fuller details in a further paper. This preliminary work is encouraging and seems likely to lead to interesting developments.

The possibilities of devising other chromatographic - potentiometric systems seem to be considerable. Many of the modern types of ion-selective electrode and the latest developments, namely selectrodes[16] and coated-wire electrodes,[17] may be suitable as micro-sensors. These sensors do not need to be highly selective in instances when there has been some prior chromatographic separation. Concerning chromatographic separations for anions, many have already been described in the literature of ion-exchange chromatography and could be adapted on a micro-scale. Thin-layer chromatography is a particularly fruitful source of separation systems, especially those where the anions of interest have high R_F values, as adaptations of such systems lead to short analysis times in column chromatography. The cadmium acetate system described under Applications was developed from such a thin-layer chromatographic method.[13]

Conclusions

The characteristics of the silver - silver chloride electrode in a flowing solution have been established. The potential is very stable under conditions of constant flow. Small changes in potential and hence small changes in the concentrations of halides can be measured.

The combination of chromatographic separation and potentiometric detection has been successfully applied to the determination of amounts of chloride of the order of nanograms in the presence of certain interfering anions and these applications offer attractive alternatives to existing methods.

An advantage of the technique is that it is suitable for rapid, repetitive analysis and, in particular, it is easily adapted to continuous monitoring.

Although the work with other electrodes and chromatographic systems has been rather limited, it has indicated that the technique should be capable of extension to the detection and determination of many other anions.

Permission to publish this paper has been given by the British Petroleum Company Limited. The authors thank Mr. A. H. Jarratt for his experimental work and the design of the instrumentation.

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Received *January* 18*th*, 1973
Amended *December* 28*th*, 1973
Accepted *February* 22*nd*, 1974

Analyst, August, 1974, Vol. 99, *pp.* 515–518

A Method for the Determination of Total Sulphur in Silicate Rocks

BY J. M. MURPHY AND G. A. SERGEANT

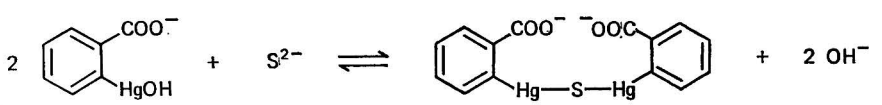
(Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ)

After oxidising sulphides to sulphates with a mixture of sodium chlorate and hydrochloric acid, the sample is refluxed with a reducing mixture of sodium iodide, red phosphorus, hypophosphorous acid, orthophosphoric acid and propionic acid. The hydrogen sulphide generated is absorbed in potassium hydroxide solution and titrated against 2-(hydroxymercuri)benzoic acid solution with dithizone as indicator. The approximate range covered is 5 to 2000 mg kg^{-1} of sulphur in the rock.

THE sulphur content of common silicate rocks is usually well below 1000 mg kg^{-1} and is therefore too low to be accurately determined gravimetrically as barium sulphate, following decomposition of the rock by oxidising fusion, as in the conventional procedure, or by wet decomposition.[1] Combustion techniques have been used in which the sample is heated, either with or without a flux such as vanadium(V) oxide, and the sulphur evolved as oxides of sulphur is determined by titration or else spectrophotometrically. This latter type of method is fairly rapid, but temperatures as high as 1450 °C appear to be required and recoveries of sulphur are not reliably quantitative.[2] A sensitive method involving the use of wet reduction has recently been described[3] in which the sample is heated at 280 °C with a mixture of anhydrous orthophosphoric acid and tin(II) chloride. Sulphur, present either as sulphide or sulphate, is evolved as hydrogen sulphide, which is then determined spectrophotometrically.

In the proposed method sulphur is recovered from the sample as hydrogen sulphide by heating with a reducing mixture modified from that used by Johnson and Nishita,[4] comprising sodium iodide, red phosphorus, hypophosphorous acid, orthophosphoric acid and propionic acid. The solid iodide is more convenient to use than hydriodic acid, and as a diluent propionic acid has been selected in preference to formic or acetic acids on account of its higher boiling-point. Red phosphorus, in addition to contributing to the reducing action, is a very effective anti-bumping agent. It is necessary to oxidise resistant sulphides, of which the commonest is pyrite, by a preliminary oxidation to sulphate. This oxidation is effected by heating the powdered sample with a mixture of sodium chlorate and concentrated hydrochloric acid. On evaporating the mixture to dryness, the solid residue includes an excess of sodium chloride, which provides bulk and thus facilitates the quantitative transfer of the residue to the distillation flask.

In an earlier version of the proposed method the hydrogen sulphide generated by reduction was determined by a colorimetric methylene blue finish similar to that described by Johnson and Nishita. This finish, although very sensitive, had a very restricted range and was limited in accuracy by the spectrophotometric properties of methylene blue solutions, even when modified by the addition of pyridine[5] in an attempt to improve linearity. A titrimetric finish was next attempted and promising results were obtained by using mercury(II) acetate as titrant with dithizone as indicator. The sensitivity of the method was retained and the range covered was extended, but it tended to be limited by the obscuring effect of the dark colour of mercury(II) sulphide on the end-point of the titration. This defect has now been eliminated by changing the titrant from mercury(II) acetate to 2-(hydroxymercuri)-benzoic acid, first proposed as a titrant for sulphide by Wroński.[6] In the reaction—



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the reaction products are colourless and a good end-point is obtained up to the recommended limit for the method of 2000 mg kg^{-1} of sulphur in the rock sample. Above this limit a gravimetric procedure is usually to be preferred.

METHOD

REAGENTS—

Analytical-reagent grade materials should be used whenever possible.

Sodium chlorate.

Hydrochloric acid, concentrated, sp. gr. 1·18—Aristar or equivalent grade.

Sodium iodide.

Phosphorus, red, amorphous.

Hypophosphorous acid, 50 *per cent.* m/m.

Orthophosphoric acid, 88 *per cent.* m/m.

Propionic acid.

Potassium hydroxide solution, 0·2 N.

Dithizone indicator—Prepare a 1 per cent. *m/m* mixture of dithizone with potassium nitrate.

2-(*Hydroxymercuri*)*benzoic acid titrant*—Dissolve 0·200 g of the acid in 1 litre of 0·2 N potassium hydroxide solution. One millilitre of this solution is approximately equivalent to 10 μg of sulphur.

Standard sulphate solution—Dissolve 1·0872 g of dry potassium sulphate in water and dilute the solution to 100 ml. This solution contains 2000 $\mu g\ ml^{-1}$ of sulphur. From it prepare, by dilution with propionic acid, a dilute standard solution containing 200 $\mu g\ ml^{-1}$ of sulphur.

APPARATUS—

The apparatus is shown in Fig. 1. A three-necked 100-ml flask is fitted with a reflux condenser, an inlet tube connected through a gas flow meter to a supply of nitrogen, and a stopper in the third neck that is used for the introduction of solid material into the flask. This stopper should be held by a retaining spring or weight to keep it firmly in position during the refluxing operation. The condenser is connected at the exit end by about 20 cm of clear PVC tubing (3 mm o.d.) to a length of 5 mm o.d. glass tubing that acts as a bubbler in the

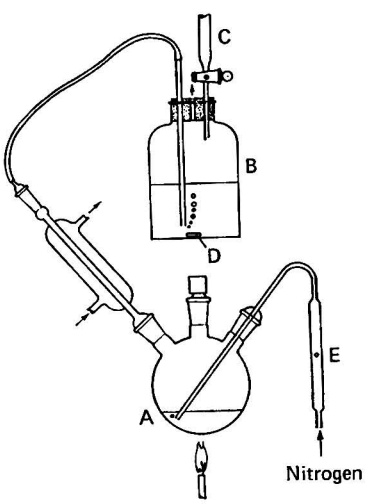

Fig. 1. Reduction and titration apparatus: A, 100-ml distillation flask; B, absorption vessel (150 ml); C, 50-ml burette; D, magnetic stirrer follower; and E, gas flow meter (5 to 190 ml min^{-1} air)

absorption vessel; the latter has a capacity of 150 ml. The tip of a 50-ml burette also passes through the bung in the absorption vessel and a third hole in the bung serves as an outlet for nitrogen.

PROCEDURE—

Mix 0·2000 g of sample powder (sieved to pass 90 mesh) and 0·20 g of sodium chlorate in a 50-ml PTFE beaker, add 5 ml of hydrochloric acid and cover the beaker with a watch-glass. Allow it to stand overnight, then place the beaker, still covered, on a steam-bath. After 30 minutes rinse down the watch-glass and sides of the beaker with a small amount of water and evaporate the contents of the beaker to dryness under an infrared lamp. Break up any large lumps of solid with a small spatula and, if the residue is not to be used at once, store the beaker in a desiccator.

To the dry 100-ml flask (Fig. 1) add 1 g of sodium iodide, 0·2 g of red phosphorus, 2 ml of hypophosphorous acid, 8 ml of orthophosphoric acid and 10 ml of propionic acid. Connect the flask to the remainder of the apparatus, as shown in Fig. 1, and pass nitrogen through the apparatus at a rate of 60 ml min^{-1} with the tubing to the absorption vessel disconnected. Turn on the condenser cooling water and, by heating the flask with a small flame protected from draughts, reflux the contents for 30 minutes in order to remove traces of sulphur from the reagent mixture. Remove the burner without interrupting the flow of nitrogen and allow the flask to cool for 15 minutes before proceeding to the next stage. The reducing mixture thus prepared can be used for about twelve successive determinations before needing replacement.

To the absorption vessel containing a magnetic stirrer follower, add 50 ml of potassium hydroxide solution and about 10 mg of dithizone indicator. Reconnect the tubing from the condenser to the absorption vessel, switch on the magnetic stirrer and add 2-(hydroxymercuri)-benzoic acid titrant dropwise from the burette until the indicator changes colour from yellow to pink. With the aid of a small, wide-necked funnel and a small, stiff brush introduce the residue from the PTFE beaker into the distillation flask, replacing the stopper without delay. Replace the burner and reflux the contents of the distillation flask for a further 30 minutes, then, after allowing the flask to cool for 10 minutes, add titrant from the burette and note the volume required to restore the pink colour. Correct for the small blank, the value of which is determined by carrying out the whole procedure without the sample. Finally, calculate the sulphur content of the sample after standardising the titrant against 1 or 2 ml of dilute standard sulphate solution, which are added directly to the distillation flask and taken through the reducing operation. The standard titration should be corrected for the blank represented by the equivalent volume of pure propionic acid added to the distillation flask and taken through the same operation.

RESULTS

Eight U.S. Geological Survey Standard rocks have been analysed for sulphur by the method and the results are shown in Table I. Comparative results by other workers can be

TABLE I

SULPHUR CONTENTS OF SOME STANDARD ROCKS

Rock	Sulphur content by proposed method/ mg kg^{-1}	Sulphur content by other methods/ mg kg^{-1}
Granite G-1	70, 60	40,[3] 58,* 175,[9] 74[10]
Diabase (dolerite) W-1	130, 135	120,[3] 123,* 135,[9] 124[10]
Granite G-2	100, 95	24,* 100†
Granodiorite GSP-1	360, 360	162,* 400†
Andesite AGV-1	20, 20	<10,* 100†
Peridotite PCC-1	20, 20	<10,* 100†
Dunite DTS-1	15, 15	<10,* 0†
Basalt BCR-1	420, 420	392,* 400†

* Average values reported by Flanagan.[7]
† Average values reported by Abbey.[8]

seen to be sufficiently widely divergent to indicate the difficulty of low-level sulphur determination in rocks. The proposed method has also been applied, with results of satisfactory precision for routine determinations, to a wide range of silicate rocks with sulphur contents from 5 to about 2000 mg kg^{-1}.

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Received *February* 19*th*, 1974
Accepted *March* 20*th*, 1974

Analyst, August, 1974, Vol. 99, *pp.* 519–522

A Titrimetric Method for the Determination of Sulphate in Fertilisers

BY A. D. CAMPBELL, D. P. HUBBARD AND N. H. TIOH

(*Department of Chemistry, University of Otago, Box 56, Dunedin, New Zealand*)

A method is described for the determination of sulphate in fertilisers in which the sulphate is precipitated with barium chloride from an acidified EDTA solution. The precipitate is filtered off by using membrane filters and is dissolved in ammoniacal EDTA; the excess of EDTA is then titrated against a solution of magnesium ions with Eriochrome black T as the indicator. No interference is encountered from iron, aluminium, fluoride or phosphate ions.

THE fertiliser industry has long been interested in accurate, rapid and simple methods for the determination of sulphate in superphosphate fertilisers. The procedure most often used is gravimetric, involving the precipitation of barium sulphate. However, this method is time consuming and subject to interferences.[1] The direct titrimetric methods for sulphate determination suffer from interferences from phosphate, iron and aluminium, etc., and require extra separation stages, which lengthen the time taken for analysis.

Excellent reviews of both gravimetric[1] and titrimetric methods[1,2] have been given. Belcher, Gibbons and West[3] reported an indirect titrimetric method, which entails the precipitation of barium sulphate, its dissolution in ammoniacal EDTA and finally back-titration with a solution of magnesium ions. Subsequently, this basic method has been successfully applied to the determination of sulphur in steel[4] and organic compounds[5] and of sulphate in urine.[6] It seemed to us that this procedure could well be adapted to the determination of sulphate in fertilisers. With minor modifications to the established method we have been able to analyse both actual and synthetic superphosphate samples with excellent accuracy and precision.

EXPERIMENTAL

REAGENTS AND SOLUTIONS—

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

Ammonia solution, sp. gr. 0·88.

Barium chloride solution, 5 per cent. m/V.

Standard magnesium chloride solution, 0·025 M—Dissolve 5·08 g of magnesium chloride, $MgCl_2.6H_2O$, in distilled water and dilute to 1 litre with distilled water. Standardise the solution against 0·04 M EDTA solution with Eriochrome black T as indicator.

EDTA solution, 0·04 M—This and other EDTA solutions at different concentrations, together with various concentrations of dilute hydrochloric and sulphuric acids, were prepared by suitable dilution of Volucon standard volumetric solution concentrates obtained from May and Baker Ltd.

Buffer solution, pH 10—Dissolve 70·0 g of ammonium chloride in a mixture of 250 ml of water and 570 ml of ammonia solution (sp. gr. 0·88), and dilute the solution to 1 litre.

Indicator solution—Dissolve 0·40 g of Eriochrome black T in a mixture of 30 ml of triethanolamine and 10 ml of absolute ethanol.

APPARATUS—

Circles of Metricel filter (25 mm in diameter, 0·45 μm pore size) were cut from sheets of filter obtained from Gelman Instruments Co., Ann Arbor, Michigan, U.S.A. In use, the filters were clamped in a demountable Pyrex microanalysis filter holder (Millipore Corp., Bedford, Mass., U.S.A.) that incorporated a glass funnel of 15 ml capacity and a filter area of approximately 2·5 cm^2.

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The tip of the burette was coated with picene wax, which was then drawn out with a wire to give a small orifice, thus allowing the delivery of smaller drops.

PREPARATION OF SYNTHETIC SUPERPHOSPHATE SAMPLES—

In view of the unavailability of standard superphosphate fertiliser samples of accurately known sulphate content, and in order to assess carefully the accuracy of the method, synthetic superphosphate solutions were prepared as follows by the method recommended by P. J. Gallaher (personal communication). Weigh out 0·62 g of Christmas Island rock (Note 1) and add an accurately known volume of 0·500 M sulphuric acid [between 6 and 8 ml (Note 2)]. Warm the mixture for a few minutes in order to complete the reaction. Add 10 ml of concentrated hydrochloric acid and heat nearly to dryness, then re-dissolve the residue in 100 ml of 2 M hydrochloric acid and filter the solution through a membrane filter. Dilute the filtrate to 250 ml with distilled water, take a 25-ml aliquot and continue as described in the method recommended below.

RECOMMENDED PROCEDURE FOR THE DETERMINATION OF SULPHATE IN SUPERPHOSPHATE—

Dissolve 1·00 g of finely powdered superphosphate (ground to pass through a 250-mesh sieve) in 10 ml of concentrated hydrochloric acid and evporate the solution nearly to dryness. Re-dissolve the residue in 100 ml of 2 M hydrochloric acid and filter the solution through a 0·45-μm membrane filter, rinsing thoroughly with distilled water. Transfer the filtrate into a 250-ml calibrated flask and dilute it to the mark with distilled water. Transfer a 25-ml aliquot into a Taylor flask and add to it 10 ml of 0·05 M EDTA solution.

Boil the solution for about 2 minutes and then add 100 ml of 0·05 M hydrochloric acid. Allow it to stand for a few minutes and slowly add 50 ml of 5·0 per cent. barium chloride solution. Then, after allowing it to stand for 5 to 10 minutes, filter the precipitate on a 0·45-μm membrane filter (Note 3) and wash it with 25 ml of 0·05 M hydrochloric acid, followed by 50 ml of distilled water. Transfer the precipitate to the original flask, using forceps to handle the filter, ad rinse any barium sulphate remaining on the glass funnel into the flask with distilled water. Add 20 ml of 0·04 M EDTA and 4 ml of ammonia solution (sp. gr. 0·88). Dilute the mixture to about 150 ml with distilled water and heat it for 15 minutes. Finally, allow it to cool, add 5 ml of buffer solution and titrate against standard 0·025 M magnesium chloride solution with Eriochrome black T as the indicator.

NOTES—

1. The choice of this rock ensures that the main possible interferences are likely to be present in relatively high concentrations. The only possible difference between this type of synthetic superphosphate and the commercial product could be a higher fluoride level as a result of its reduced evolution during reaction. However, only a small proportion of the fluoride is evolved from Christmas Island rock during superphosphate manufacture so that the standard solution will not be very different from that of a commercial product.

2. The stated volume of sulphuric acid ensures that the amount of sulphate in the synthetic superphosphate samples closely resembles the amount in actual samples, *i.e.*, of the order of 30 per cent. of sulphate (10 per cent. as sulphur). In addition, the total amount of added sulphate *plus* rock is close to 1 g, which again agrees with the recommended mass of sample for commercial superphosphate in the method that follows.

3. It is known that aged barium sulphate is much more difficult to dissolve than the freshly precipitated variety.[5] However, precipitation in the cold and the short time of standing produce a very fine precipitate, hence the use of the fine pore filter. This precipitate dissolves readily in ammoniacal EDTA.

RESULTS AND DISCUSSION

It was to be expected that ions such as PO_4^{3-}, F^-, Fe^{3+} and Al^{3+}, which interfere in the precipitation of barium sulphate,[7] would also interfere in the proposed titrimetric method. All of these ions are likely to be present in superphosphate samples. However, in preliminary experiments, we found no evidence of interference from either phosphate (11·5 mg) or fluoride (0·95 mg) in the determination of sulphate (12·0 mg) when following the recommended procedure with the slight modification that the initial precipitation was carried out in the absence of EDTA, *i.e.*, basically the method of Belcher *et al.*[3] However, in the presence of phosphate (23·0 mg), both iron (3·52 mg) and aluminium (1·70 mg) gave a positive error of

2·4 per cent. in the determination of 24·0 mg of sulphate. A solution containing phosphoric acid, sodium fluoride, iron(III) chloride or aluminium chloride was added to a standard volume of sulphuric acid and subjected to the described procedure in studies of these interferences.

Přibil and Maricova[8] have shown that if barium sulphate is precipitated in the presence of EDTA, then the co-precipitation of moderate amounts of many ions is prevented. Modification of the procedure accordingly eliminated the interference of iron(III) ions; however, a slight interference from aluminium remained. It is well known that aluminium complexes only slowly with EDTA but that boiling speeds up the reaction[9,10]. The time of boiling needed to complete complexation is dependent on several factors, such as pH and previous treatment of the solution.[9] Milner and Woodhead[10] recommend boiling for about 2 minutes and the aluminium interference was eliminated under these conditions.

The results obtained all show a very small positive bias, which may or may not be significant. This bias is not serious.

During the development of the method it was noted that phosphate caused an interference in the determination when the precipitation of barium sulphate was carried out in the presence of EDTA. This interference was eliminated by washing the precipitate with dilute hydrochloric acid. It was also necessary to increase the amount of barium chloride used in order to achieve the complete precipitation of barium sulphate when EDTA was present. Washing the precipitate with EDTA in addition to dilute acid made no difference to the recovery of sulphate and was therefore discontinued.

TABLE I

ANALYSIS OF SYNTHETIC SUPERPHOSPHATE SOLUTIONS

Amount of sulphate added/mg*	Amount of sulphate recovered/mg†	Mean recovery, per cent.
317·0	317·7, 317·7, 317·3, 316·8	100·1
369·9	371·0, 370·5, 371·4, 370·5	100·3
317·0	317·7, 317·7, 317·7, 316·8	100·1
313·2	314·7, 314·2, 313·8, 313·8	100·3

* Amounts added to approximately 0·625 g of Christmas Island phosphate rock.

† Four separate, 25-ml aliquots of each synthetic superphosphate stock solution were taken through the recommended procedure.

Much has been written about the suitability of Eriochrome black T as an indicator.[11] We have found that the final colour change to pink is, in fact, very distinct against a white background, and that reproducible titrations can be obtained. In Table I the recoveries of sulphate added to Christmas Island rock that had been shown to contain no natural sulphate are shown. The accuracy of the method is within that required by the fertiliser industry (within 0·5 per cent.) and the precision is excellent. In Table II the results obtained for four actual superphosphate fertiliser samples are given.

TABLE II

ANALYSIS OF ACTUAL SUPERPHOSPHATE SAMPLES

Sample number	Nominal sulphur content, per cent.*	Sulphur content determined, per cent.
1	11·17	11·20, 11·19, 11·21
2	10·16	10·18, 10·17, 10·18
3	10·42	10·45, 10·45, 10·46
4	11·11	11·12, 11·13, 11·13

* Values supplied by The New Zealand Fertiliser Manufacturers' Research Association, obtained by use of a calibrated gravimetric procedure.

CONCLUSIONS

It has been shown, via the analysis of both actual and synthetic superphosphate solutions, that the indirect method, first proposed by Belcher *et al.*,[3] for the determination of sulphate can, with modification, be applied to sulphate in phosphate fertilisers. The method involves the

precipitation of the sulphate with barium chloride from an acidified EDTA solution to prevent the co-precipitation of various ions. The precipitate is filtered off and dissolved in ammoniacal EDTA and the excess of EDTA titrated against standard magnesium solution with Eriochrome black T as the indicator. Excellent agreement with available data for the actual samples and virtually 100 per cent. recoveries of sulphate for the synthetic samples demonstrate the reliability of this procedure. The method is straightforward, relatively fast (several samples could be analysed simultaneously within 1 hour) and accurate.

The authors thank the Research Committee of the New Zealand Grants Committee, for financial assistance, and also Mr. P. J. Gallaher, of the New Zealand Fertiliser Manufacturers' Research Association, for his valuable advice.

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Received *February 8th*, 1974
Accepted *March 8th*, 1974

Analyst, August, 1974, Vol. 99, *pp.* 523–527

Loss of Zinc and Cobalt During Dry Ashing of Biological Material

BY J. G. VAN RAAPHORST, A. W. VAN WEERS AND H. M. HAREMAKER

(*Reactor Centrum Nederland, Petten, The Netherlands*)

Loss of zinc and cobalt during dry ashing of marine mussels (*Mytilus edulis*) and brown seaweed (*Fucus spiralis*) has been studied by using material labelled by exposure of living organisms to sea water spiked with zinc-65 or cobalt-60. Even after ashing in porcelain crucibles at temperatures of up to 1000 °C, no significant loss of zinc or cobalt by volatilisation was observed. After ashing at 450 and 550 °C, both radionuclides could be removed quantitatively from the crucibles by leaching with hydrochloric acid. Adsorption on the crucible after ashing at 1000 °C was measured only for cobalt-60. A significant proportion of the cobalt tracer could not be removed from the crucibles by treatment of the latter with acid. From these results it is concluded that dry ashing is a reliable method of sample destruction for the determination of zinc and cobalt in *M. edulis* and *F. spiralis*.

IN studies on the levels of trace metals in biological samples, both wet and dry-ashing techniques are used for the destruction of the organic material in order to bring the samples into a condition suitable for analysis. Dry ashing has the advantage of being a relatively simple method that gives a large reduction in sample volume without the risk of contamination with impurities that may be present in oxidising acids.

In our laboratory a study was made of the seasonal and long-term variation of the levels of zinc and cobalt in marine mussels and seaweed,[1,2] and for the reason given above dry ashing was regarded as the most suitable method of sample destruction. However, during this procedure losses of trace metals may occur. The behaviour of the elements zinc and cobalt during dry ashing of organic material is the subject of contradictory reports in the literature. In his book, Gorsuch[3] published data obtained from recent literature, which showed that the recovery of zinc varied considerably. Gorsuch[4] measured the amount of zinc retained after ashing neutron-activated human hair at 500 °C for 16 hours and repeated the measurements after further 3-hour periods of ashing at 600, 700, 800, 900 and 1000 °C. The results obtained showed that only at 1000 °C may loss of a few per cent. of zinc by volatilisation occur.

More serious was the retention of zinc on the silica of the crucible, which increased from 0·3 per cent. after 3 hours at 600 °C to 7·1 per cent. after 3 hours at 1000 °C. The results given by Gorsuch indicate further that the adsorption of zinc on crucibles increases considerably with the ashing time. In contrast with these results, Pyck, Hoste and Gillis[5] reported a zinc recovery of only 30 per cent. after ashing for 3 hours at 900 °C. Results published by other investigators are no less conflicting. Hamilton, Minski and Cleary[6] observed that no loss of zinc occurred at 850 °C but found that 45 per cent. of it was adsorbed on the silica of the crucible. Doshi, Sreekumaran, Mulay and Patel[7] reported a 66 per cent. recovery after ashing for 24 hours at 700 °C, Knauer[8] observed that there was no loss at 800 °C and Strohal, Lulic and Jelisavčič[9] obtained only a 56 per cent. recovery of zinc at 800 °C.

Published data concerning the loss of cobalt during dry ashing are also contradictory. Gorsuch[4] claimed a 99 to 100 per cent. recovery of cobalt and that there was no significant retention of it on the crucible. Pyck *et al.*[5] reported a recovery of 70 per cent. after ashing for 3 hours at 900 °C, Doshi *et al.*[7] found that 64 per cent. was recovered after 24 hours at 700 °C while Strohal *et al.*[9] reported a recovery of 79 per cent. even after ashing at a temperature as low as 350 °C.

In some of the studies mentioned above,[4–6] radioactive tracers were simply added to the samples immediately before ashing them. However, one can expect the radioactive and non-radioactive molecules to have the same chemical structure only if the radioactive tracer is incorporated in a more or less natural way, as effected for instance by Strohal *et al.*[9] Neutron

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irradiation of the organic material prior to ashing it, as carried out by Gorsuch,[3] may cause great differences in the chemical structure between the activated and the stable molecules.

It is therefore clear from the foregoing results obtained with different material and by using different methods that no general conclusion on the recovery of zinc and cobalt after dry ashing can be drawn. Moreover, several of these studies apparently involved only a small number of independent determinations.

In the present study, marine mussels and seaweed were labelled by exposure of living organisms to sea water spiked with radioactive zinc or cobalt. Each treatment of the labelled material was carried out on a series of replicate samples.

Experimental

Labelling of mussels and seaweed—

Mussels (*Mytilus edulis*) and brown seaweed (*Fucus spiralis*) were collected from the North Sea shore near Petten. Up to fifteen mussels were placed in a 30-litre polyethylene tank filled with filtered natural sea water and to the tank zinc-65 or cobalt-60 was added at a concentration that varied from 1 to 10 μCi l^{-1}. Labelling of seaweed was carried out in 2-litre Erlenmeyer flasks with filtered natural sea water, buffered at pH 8 with 0·02 M Tris(hydroxymethyl)aminomethane hydrochloride, and zinc-65 or cobalt-60 was added at a concentration of 1 to 3 and 4 to 8 μCi l^{-1}, respectively. Each flask contained 20 to 40 g of fresh seaweed. The radioisotopes used were of high specific activity and were obtained commercially in the chloride form from the Radiochemical Centre, Amersham.

After a labelling period of 5 days for seaweed and 5 to 10 days for mussels, the seaweed and the soft parts of the mussels were either dried overnight in an oven at 45 °C or freeze-dried. The method of drying was shown to have no influence on the results of the experiments.

Ashing procedure—

Ashing was carried out in a Solo, Type 8003, furnace, and the temperature control and indicator were checked with a thermocouple. Each sample was ashed in a Staatliche Berlin, Type C/O, porcelain crucible successively at 200, 300, 400, 600, 800 and 1000 °C for 20 hours at each temperature. Each sample remained in the same crucible from the beginning until the end of the whole procedure, including the counting procedure. Nine samples (200 to 400 mg dry mass) from each organic material were ashed at the same time.

Counting procedure—

Counting was performed with a 3 × 3-inch thallium-activated sodium iodide crystal, connected to a single-channel analyser.

The samples in the procelain crucibles were counted in a fixed geometry at a distance of 18 cm from the top of the crystal and the whole counting procedure was repeated twice with each sample. A total of about 100 000 counts was recorded for each sample. In order to ensure that a distance of 18 cm was sufficient to prevent changes in geometry that were too large from occurring during ashing, an experiment was carried out so as to check this distance.

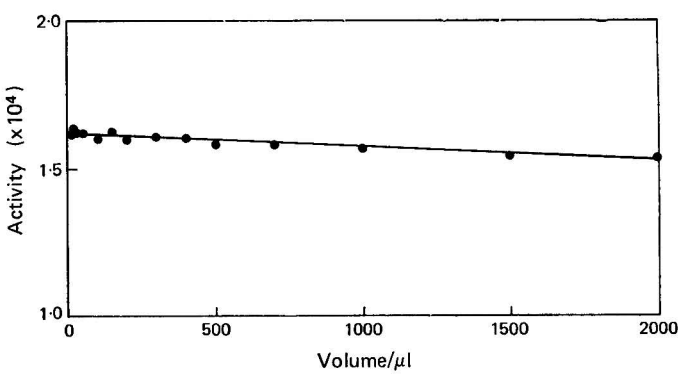

Fig. 1. Influence of sample volume on counting rate

A volume (10 μl) of a solution containing zinc-65 was placed in a crucible and counted at a distance of 18 cm from the crystal. To this solution 20 μl of water were then added and the solution was counted again. This procedure was repeated with successive additions of 20 to 500 μl of water up to a final sample volume of 1500 μl. The results given in Fig. 1 show that when the volume of the sample is kept below 500 μl the influence of sample volume on the counting rate is less than 1·5 per cent.

TABLE I

ACTIVITY OF ZINC-65 IN MUSSELS AND SEAWEED AFTER ASHING PROCEDURE

Results, which are the means of nine determinations, are expressed as counts per 1000 s per 100 mg of original dry mass

Ashing temperature/ °C	Mussels		Seaweed	
	Activity	Standard deviation	Activity	Standard deviation
—	18·783	0·231	12·551	0·145
200	19·105	0·211	12·632	0·173
400	19·527	0·207	12·988	0·166
500	19·493	0·230	—	—
600	19·741	0·201	12·795	0·144
800	19·726	0·157	12·491	0·120
1000	19·813	0·232	12·625	0·104
Mean	19·455		12·680	
Standard deviation ..	0·379		0·182	

LEACHING—

Several workers have reported difficulties experienced with the complete removal of the ash residue from silica crucibles. We checked this aspect for porcelain crucibles by ashing samples of labelled material at 450, 550 and 1000 °C, each sample being subsequently placed on top of the crystal and counted. The crucibles were then rinsed with water and the counting was repeated. When activity was detected in the crucible, approximately 1 ml of 10 N hydrochloric acid was added and the mixture was carefully evaporated to dryness. Finally, about 3 ml of 0·1 N hydrochloric acid were added, the crucible was heated gently, rinsed subsequently with water and counted again.

TABLE II

ADSORPTION OF ZINC ON PORCELAIN CRUCIBLES AFTER ASHING OF MUSSELS AND SEAWEED AND SUBSEQUENT LEACHING

Results are adsorbed activity expressed as percentage of the activity of the ashed sample

Mussels				Seaweed			
Ashing temperature 450 °C; leached with		Ashing temperature 550 °C; leached with		Ashing temperature 450 °C; leached with		Ashing temperature 550 °C; leached with	
water	acid	water	acid	water	acid*	water	acid
0·2	<0·1	0·1	<0·1	0·2	—	2·4	1·4
0·2	<0·1	0·3	<0·1	0·5	—	1·1	0·3
0·2	<0·1	0·6	<0·1	0·2	—	1·2	0·1
0·3	<0·1	0·6	<0·1	<0·1	—	1·3	1·0
0·3	<0·1	0·7	0·4	0·4	—	0·7	0·4
0·2	<0·1	1·2	0·2	<0·1	—	0·9	0·3
0·1	<0·1	0·4	<0·1	0·1	—	1·2	0·6
0·3	<0·1	0·3	<0·1	<0·1	—	1·0	0·7
0·2	<0·1	0·9	0·4	<0·1	—	5·7	3·4

* Not measured.

RESULTS AND CONCLUSION

Unless indicated otherwise, the measured activities of the samples were expressed as counts per 1000 s per 100 mg (original dry mass) of the material.

ZINC—

The results of the ashing procedure are given in Table I. For mussels the activities measured initially and after ashing at 200 °C are somewhat lower than the values obtained after ashing at higher temperatures, which may be explained by a geometry factor, because at temperatures above 400 °C the volume of the samples becomes very small. It is clear that loss of zinc by volatilisation does not occur even at 1000 °C.

Adsorption of zinc on porcelain crucibles was measured after ashing the material at 450 and 550 °C. The very small amounts that remained adsorbed on the crucible after leaching (Table II) show that essentially all of the zinc can be removed from the crucible with the aid of hydrochloric acid.

TABLE III

ACTIVITY OF COBALT-60 IN MUSSELS AND SEAWEED AFTER ASHING PROCEDURE

Results, which are the means of eight determinations for mussels and nine for seaweed, are expressed as counts per 1000 s per 100 mg of original dry mass

Ashing temperature/ °C	Mussels		Seaweed	
	Activity	Standard deviation	Activity	Standard deviation
—	16·170	0·336	27·440	0·266
200	15·776	0·476	27·448	0·255
300	16·101	0·505	27·389	0·257
400	15·711	0·409	27·396	0·228
600	16·083	0·480	27·512	0·278
800	16·027	0·460	27·629	0·312
1000	15·610	0·488	27·562	0·300
Mean	15·925		27·482	
Standard deviation ..	0·221		0·89	

COBALT—

Ashing of mussels and seaweed at temperatures of up to 1000 °C causes no losses of cobalt, as can be seen from the results given in Table III. Only very slight adsorption of cobalt on the crucible was observed after ashing at 450 and 550 °C (Table IV) and leaching with both water and hydrochloric acid removed it quantitatively. Ashing at 1000 °C resulted in a melt being formed, which could not be removed from the crucible with water. Subsequent

TABLE IV

ADSORPTION OF COBALT ON PORCELAIN CRUCIBLES AFTER ASHING OF MUSSELS AND SEAWEED AND SUBSEQUENT LEACHING

Results are adsorbed activity expressed as percentage of the activity of the ashed sample

Mussels						Seaweed					
Ashing temperature 450 °C; leached with		Ashing temperature 550 °C; leached with		Ashing temperature 1000 °C; leached with		Ashing temperature 450 °C; leached with		Ashing temperature 550 °C; leached with		Ashing temperature 1000 °C; leached with	
water	acid	water	acid	water	acid	water	acid*	water	acid	water	acid
0·2	<0·1	0·1	<0·1	101·3	10·5	<0·1	—	<0·1	—*	98·5	1·7
0·2	<0·1	0·1	<0·1	79·2	6·8	<0·1	—	<0·1	—*	99·0	1·9
0·2	<0·1	0·2	<0·1	99·4	9·0	<0·1	—	<0·1	—*	98·0	1·4
0·2	<0·1	1·1	0·1	99·6	9·9	<0·1	—	<0·1	—*	99·7	1·9
0·1	<0·1	0·2	0·1	99·5	6·4	<0·1	—	<0·1	—*	99·4	1·8
0·2	<0·1	0·1	0·1	100·9	9·7	<0·1	—	0·1	<0·1	101·2	1·7
0·1	<0·1	0·3	0·3	74·3	17·5	<0·1	—	0·3	<0·1	98·8	1·6
0·1	<0·1	0·1	<0·1	101·1	14·1	<0·1	—	0·9	<0·1	98·7	1·5
0·1	<0·1	0·2	0·1	100·2	14·1	<0·1	—	0·2	<0·1	99·4	1·7

* Not measured.

treatment with hydrochloric acid removed the cobalt quantitatively from the crucibles containing seaweed samples, but a significant proportion (6 to 18 per cent.) of the cobalt in mussels could not be dissolved.

In conclusion it can be stated that dry ashing has been shown to be a reliable method of sample destruction for the determination of zinc and cobalt in mussels (*Mytilus edulis*) and in brown seaweed (*Fucus spiralis*). The only restriction is that after ashing at very high temperatures the quantitative removal of the residue from a porcelain crucible has proved to be difficult.

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Received *December 3rd*, 1973
Accepted *February 18th*, 1974

Analyst, August, 1974, Vol. 99, *pp.* 528–532

Determination of Copper(I) with *N*-Bromosuccinimide

BY A. ABOU EL KHEIR, M. AYAD* AND M. M. AMER

(*Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt*)

It has been found that *N*-bromosuccinimide readily and quantitatively oxidises aqueous solutions of copper(I) at room temperature and in the presence of dilute hydrochloric acid, the oxidising agent being irreversibly reduced to succinimide.

A procedure is suggested for the determination of copper(I) by titration with standard *N*-bromosuccinimide solution; the results obtained were found to be equally precise but more accurate than those given by the permanganate and complexometric methods.

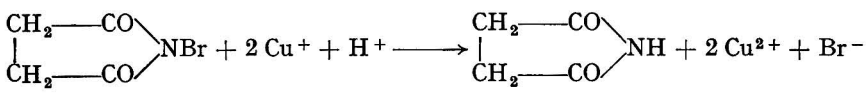
Copper(II), after preliminary reduction, can also be determined by the suggested procedure, whether alone or admixed with copper(I).

COPPER is commonly determined by complexometric, iodimetric or electrolytic procedures; however, the last of these procedures is time consuming and requires the use of much apparatus.

Flaschka[1] and Amin[2] described a direct complexometric procedure for the titration of copper(II), using murexide as indicator. Eriochrome black T has been used as an indicator in an indirect complexometric determination of copper by back-titration of excess of EDTA with a standard solution of a manganese(II) salt.[3] Other proposed indicators include pyrocatechol violet,[4] 1,2-(pyridylazo)-2-naphthol[5,6] and fast sulphone black F.[7]

Both copper(I) and copper(II) have been determined by iodimetric titration, copper(II) salts by the procedures of Lehmann,[8] Riegler,[9] Maquenne,[10] Citron,[11] Peters,[12] and Shaffer and Hartmann[13,14] and copper(I) salts by those of Bang,[15,16] Scales,[17] Maclean,[18,19] Clark[20] and Shaffer and Hartmann.[13,14]

This paper describes the use of *N*-bromosuccinimide as an oxidising agent for determining copper(I), the reaction involved being as follows—



The mechanism of the reaction has been established by experiment. However, although *N*-bromosuccinimide can decolorise methyl red in an aqueous acidic medium, it oxidises copper(I) preferentially. On adding *N*-bromosuccinimide in the presence of methyl red, the red colour of the indicator remains unchanged until all of the copper(I) ions have been oxidised, the first excess of *N*-bromosuccinimide decolorising the indicator.

EXPERIMENTAL

REAGENTS—

Standard copper(I) chloride solution, 0·005 M—Dissolve 100 g of copper(II) acetate in 1 litre of water, filter and heat the solution to boiling, then add 15 per cent. glucose solution until the blue colour of copper(II) almost disappears. Allow the mixture to stand, decant the supernatant liquid, wash the precipitate of copper(I) oxide several times with water, by decantation, then transfer it into a Gooch crucible. Wash the precipitate on the crucible with ethanol, then dry it in a vacuum desiccator. Dissolve 0·0715 g of the dried copper(I) oxide in 20 per cent. (*V*/*V*) hydrochloric acid, avoiding the use of heat, and make the solution up to 100 ml.

Methyl red solution, 0·04 per cent. (m/V) *in 95 per cent. ethanol.*

N-*Bromosuccinimide solution, 0·01* M, *aqueous*—Standardise this solution, which must be freshly prepared, against standard arsenite solution[21] and keep it in dark-glass bottles.

* Present address: Research and Control Centre, Cairo, Egypt.

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Hydrochloric acid, 20 per cent. V/V—Mix 1 volume of concentrated hydrochloric acid and 4 volumes of water.

PROCEDURES—

1. *Determination of copper(I) alone*—Introduce into a 50-ml conical flask an accurately measured volume of the copper(I) salt solution (containing 5 to 20 mg of Cu), an equal volume of 20 per cent. *V/V* hydrochloric acid and two drops of methyl red indicator solution, and titrate the mixture by the dropwise addition of 0·01 M *N*-bromosuccinimide solution, while stirring, until the red colour is just discharged. Carry out a blank experiment (1 ml of 0·01 M *N*-bromosuccinimide solution corresponds to 1·27 mg of Cu, 1·43 mg of Cu_2O and 1·98 mg of Cu_2Cl_2).

TABLE I

DETERMINATION OF COPPER(I) CHLORIDE BY TITRATION WITH *N*-BROMOSUCCINIMIDE SOLUTION

Copper(I) chloride taken/ mg	Copper(I) chloride found/ mg	Recovery, per cent.
1·98	1·96	99·0
2·97	3·06	103·0
3·96	3·98	100·5
4·95	4·94	99·8
9·90	9·77	98·7
14·85	14·73	99·2
19·80	19·53	98·6
24·75	24·25	98·0
	Mean ($P = 0·05$)	99·6 ± 1·25

2. *Determination of copper(II) alone and of total copper*—Neutralise a volume of the copper(II) salt solution (equivalent to 5 to 20 mg of Cu) with a few drops of 10 per cent. sodium hydrogen carbonate solution until effervescence ceases. Boil the solution, add saturated glucose solution, dropwise, until the blue colour completely disappears, then continue boiling the solution for a further 2 minutes. Filter it through a Gooch crucible, washing the precipitate with water by decantation.

Dissolve the washed precipitate of red copper(I) oxide on the filter in about 5 ml of concentrated hydrochloric acid, dilute the solution with 20 ml of water, add 3 to 5 drops of methyl red indicator and titrate it with 0·01 M *N*-bromosuccinimide solution. Carry out a blank experiment (1 ml of 0·01 M *N*-bromosuccinimide solution corresponds to 1·270 mg of Cu and 1·590 mg of CuO).

3. *Determination of copper(II) and copper(I) in mixtures*—Determine copper(I) on a volume of the solution of the mixed ions containing the equivalent of 5 to 20 mg of copper(I)

TABLE II

DETERMINATION OF COPPER(I) CHLORIDE BY TITRATION WITH *N*-BROMOSUCCINIMIDE SOLUTION AND BY THE PERMANGANATE METHOD[22]

	N-Bromosuccinimide method		Permanganate method	
Copper(I) chloride taken/ mg	Copper(I) chloride found/ mg	Recovery, per cent.	Copper(I) chloride found/ mg	Recovery, per cent.
4·95	4·80	97·0	4·80	97·0
	4·99	101·0	4·85	98·0
	4·85	98·0	4·90	99·0
9·9	9·99	101·0	9·60	97·0
	9·80	99·0	9·60	97·0
	9·95	100·5	9·80	99·0
19·8	19·55	98·8	19·50	98·5
	19·50	98·5	19·60	99·0
	19·85	100·2	19·60	99·0
	Mean ($P = 0·05$)	99·33 ± 1·09		98·18 ± 0·64

by the method described under Procedure 1. Then determine the total copper content on a volume containing the equivalent of 5 to 20 mg of copper by the method described under Procedure 2. Calculate the copper(II) content by difference.

TABLE III

DETERMINATION OF COPPER(II) OXIDE BY THE *N*-BROMOSUCCINIMIDE PROCEDURE AND THE COMPLEXOMETRIC METHOD[23]

	N-Bromosuccinimide method		Complexometric method	
Copper(II) oxide taken/ mg	Copper(II) oxide found/ mg	Recovery, per cent.	Copper(II) oxide found/ mg	Recovery, per cent.
10	9·83	98·30	9·56	95·6
	9·83	98·30	9·78	97·8
	9·87	98·70	9·78	97·8
20	19·72	98·60	19·30	96·5
	19·87	99·35	19·30	96·5
	20·03	100·15	19·87	99·3
30	29·61	98·70	29·40	98·0
	29·70	99·00	30·00	100·0
	29·70	99·00	29·80	99·3
40	40·22	100·50	39·54	98·9
	40·06	100·20	39·54	98·9
	40·06	100·20	39·74	99·3
50	48·80	97·60	48·89	97·8
	49·29	98·60	48·89	97·8
	49·29	98·60	48·99	98·0
	Mean ($P = 0·05$)	99·1 ± 0·474		98·1 ± 0·678

RESULTS

Table I shows the results obtained by applying Procedure 1 to the determination of 2 to 25 mg of copper(I) chloride and in Table II the results obtained for the determination of 5 to 20 mg of copper(I) chloride by both the suggested *N*-bromosuccinimide procedure and the permanganate method[22] are compared. In Table III the results obtained for the determination of 10 to 50 mg of copper(II) oxide by both the *N*-bromosuccinimide procedure and the complexometric method[23] are compared and Table IV gives the results obtained by applying Procedure 3 to the determination of different mixtures of copper(I) and copper(II) oxides.

TABLE IV

DETERMINATION OF COPPER(I) AND COPPER(II) OXIDES IN MIXTURES BY THE *N*-BROMOSUCCINIMIDE PROCEDURE

Copper(I) oxide taken/ mg	Copper(I) oxide found/ mg	Recovery, per cent.	Copper(II) oxide taken/ mg	Copper(II) oxide found/ mg	Recovery, per cent.
20	20·50	102·5	20	20·10	100·5
	20·50	102·5		19·95	99·8
	20·30	101·5		19·95	99·8
20	20·30	101·5	50	48·97	97·9
	20·30	101·5		49·20	99·2
	20·16	100·8		50·80	101·6
10	10·01	100·1	50	48·80	97·6
	10·01	100·1		48·90	97·9
	10·20	102·0		49·30	98·6
50	49·90	99·8	20	19·95	99·8
	49·90	99·8		19·88	99·4
	50·10	100·2		19·77	98·9
	Mean ($P = 0·05$)	101·1 ± 1·93			99·24 ± 1·51

DISCUSSION

N-Bromosuccinimide, which contains a loosely bound bromine atom, is used for bromination as well as for dehydrogenation purposes, especially in organic chemistry.[24] However,

solutions of this reagent have been used for oxidimetric titrations of some inorganic ions,[25,26] the mechanism of the reaction with copper(I) in a dilute acidic medium being shown in the introduction.

The presence of bivalent copper ions was established by the formation of a blue precipitate of copper(II) hydroxide when sodium hydroxide was added to the titrated solution; the precipitate was dissolved in dilute hydrochloric acid and its identity confirmed by the potassium hexacyanoferrate(II) test. The presence of hydrobromic acid was confirmed by the silver nitrate and the chlorine water tests. Succinimide was isolated by distilling the clear reaction mixture under reduced pressure and recrystallising the solid residue from benzene; the colourless crystals were identified as succinimide (m.p. 124–125 °C).

Before making use of the above reaction for the quantitative determination of copper(I), the effect of the concentration of hydrochloric acid on the reaction was studied (Table V), and it was concluded that a concentration of 20 per cent. *V*/*V* was the most satisfactory; the recovery of 108 per cent. of copper(I) when 50 per cent. *V*/*V* hydrochloric acid was used is an effect of the high acid concentration, as previously observed by Barakat and Shehab.[21]

TABLE V

EFFECT OF HYDROCHLORIC ACID CONCENTRATION ON THE REACTION BETWEEN *N*-BROMOSUCCINIMIDE AND COPPER(I)

Copper(I) chloride taken/mg	Concentration of hydrochloric acid, per cent. *V*/*V*	Recovery, per cent.
9·9	2	62·4
	5	75·1
	10	87·9
	20	99·6
	30	102·0
	50	108·0

It is evident from the results given in Tables I to III that the accuracy of the proposed method amounts to between 99·3 and 99·6 per cent. for copper(I) and to 99·1 per cent. for copper(II). Statistical analysis of the results in Tables II and III, shown in Tables VI and VII, reveals that the variance ratios are within the theoretical limits, indicating that the suggested *N*-bromosuccinimide method is as precise as the permanganate method[22] and the complexometric procedure.[23] However, the *t*-test reveals that the *N*-bromosuccinimide procedure is more accurate than the other two methods; it also has the further advantage that it is less time consuming.

TABLE VI

STATISTICAL ANALYSIS OF THE RESULTS IN TABLE II

	N-Bromosuccinimide method	Potassium permanganate method
Mean, per cent. ($P = 0·05$)	99·33 ± 1·086	98·18 ± 0·64
N	9	9
Variance	1·997	1·3475
$t_{0·975}$	2·2836 (2·12)*	
Degrees of freedom	$\phi = 16$	
F	2·2865 (3·44)*	

* Theoretical values.

As shown in Table IV, the accuracy and precision of the proposed method for the determination of copper(I) were maintained in the presence of widely different proportions of copper(II).

TABLE VII

STATISTICAL ANALYSIS OF THE RESULTS IN TABLE III

	N-Bromosuccinimide method	EDTA method
Mean, per cent. ($P = 0{\cdot}05$)	$99{\cdot}1 \pm 0{\cdot}474$	$98{\cdot}1 \pm 0{\cdot}678$
N	15	15
Variance	0·733	1·497
$t_{0{\cdot}975}$	2·5937 (2·048)*	
Degrees of freedom	$\phi = 28$	
F	2·0409 (2·53)*	

* Theoretical values.

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Received *November 21st*, 1973
Accepted *March 5th*, 1974

Book Reviews

IR-THEORY AND PRACTICE OF INFRARED SPECTROSCOPY. By NELSON L. ALPERT, WILLIAM E. KEISER and HERMAN A. SZYMANSKI. Pp. xiv + 380. London and New York: Plenum/Rosetta Editions. Paperback Edition 1973. Price $9.14.

Szymanski's original book, published in 1964 by Plenum Press at a selling price of $15, was widely acclaimed. It was succeeded, in 1970, by a second edition under the authorship of Alpert, Keiser and Szymanski, and it is precisely that book which is now being offered, as a paperback edition, at a very considerable reduction in the previous price despite the inflationary increases of the past three years.

"Szymanski" is a well known, established text; the present circumstances do not justify a fresh appraisal of its contents, or of its merits and demerits. It was, however, always too expensive a book to be considered seriously for class adoption (at least by this reviewer's students); this barrier, at least, has now been removed. It is to be hoped that the price reduction will be appreciated by readers other than students and that publishers will adopt more freely this long-established way of making the more expensive texts available at more attractive prices. On the other hand, "Szymanski" has always had examples of infrared spectra housed in a pocket attached to its back cover; this imposes a strain on the binding such that the back cover of this reviewer's copy was torn at the spine when it was received. This could prove to be a false economy, therefore, for library and reference copies.

D. M. W. ANDERSON

ISOTOPE DILUTION ANALYSIS. By J. TÖLGYESSY, T. BRAUN and M. KYRŠ. *International Series of Monographs in Analytical Chemistry, Volume* 49. Pp. 194. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1972. Price £3·50.

This is the first book, so far as the reviewer knows, devoted exclusively to isotope dilution analysis, a subject which, especially in its radiochemical form, has expanded rapidly over the last decade or so.

For quantitative analysis it is necessary either to separate the element or compound to be determined quantitatively from the matrix so that it can be measured by an appropriate method, for example, by weighing, or to separate a property or characteristic, such as light absorption at a defined wavelength, from those due to the matrix, for measurement.

Where quantitative separation is impossible a quantitative result is still attainable if an accurate measurement of the recovery of the separated constituent can be made; isotope dilution can provide such a measurement.

In the first chapter the principles and theory of isotope dilution analysis in its various modifications are described. The chapter is almost exclusively devoted to methods employing radioactive isotopes, even in the section on page 16 entitled "Isotope Dilution Analysis with Stable Isotopes." There is, it is true, a short chapter (5) that deals with isotope dilution analysis using stable isotopes. Chapter 2 deals with experimental techniques of separation and mass determinations, Chapters 3 and 4 describe inorganic, organic and biochemical application and the final chapter some special applications of isotope dilution such as blood volume determination in the human body, dissocation constants and the capacity of ion exchangers. Each chapter includes an extensive bibliography.

The prominence given to the use of radioactive isotopes is a measure of, on the one hand their greater applicability, and on the other, the more ready availability of the necessary measuring equipment.

The book is, on the whole, well written, although there are features lacking clarity, for example, Table 1.5 "Illustration of some methods of double IDA" was incomprehensible to the reviewer and in the section (1.4) dealing with "Precision, accuracy and sensitivity of IDA," precision and accuracy appear to be confused. Thus, systematic errors are considered under precision. Although such errors will militate against the accuracy of a determination, that is, the nearness to the true value, they will usually have little effect on the repeatability, that is, the precision of a series of measurements.

In spite of the above strictures the reviewer considers this to be a valuable addition to the literature of analytical chemistry.

D. A. LAMBIE

ADVANCES IN RAMAN SPECTROSCOPY. Volume 1. PROCEEDINGS OF THE THIRD INTERNATIONAL CONFERENCE ON RAMAN SPECTROSCOPY, UNIVERSITY OF RHEIMS, FRANCE, SEPTEMBER 1972. Edited by J. P. MATHIEU. Pp. xiv + 639. London, New York and Rheine: Heyden & Son Ltd. 1973. Price £16; $44; DM131.50.

Since the introduction of lasers, there has been not only a revival of Raman spectroscopy, but also extensive development in its practical application and scope. Unfortunately, the proceedings of the first two International Conferences on Raman Spectroscopy, held at Ottawa (1969) and Oxford (1970), were not published, but since this extensive volume, which records all the papers presented at the third conference held at Rheims in September 1972, has been declared to be the first in a new series, the publication of the proceedings of future Raman conferences now appears to be assured.

This is an excellent production throughout. The Editor deserves immense credit for having completed his task by January 1973. Similarly, the publishers and their printers are to be congratulated on achieving publication as quickly as July 1973—a remarkable feat considering the bilingual character of the book, the very large number of diagrams, and the extent of the heavy mathematical typesetting required on many of the pages.

The texts of eighty-two papers (nineteen in French) are presented in eight sections: Non-linear Phenomena, New Techniques, Phase Transitions, Flame Raman Effect, Macromolecules and Biological Molecules, Resonance Raman Effect, Band Profiles in Gases and Liquids, and Molecular Structures. There is an index of contributors and a subject index.

Although only about 20 per cent. of the papers published in this book are of direct analytical interest, Raman spectroscopy has considerable analytical potential, and the contents of this book could be read with advantage by analytical chemists, particularly those that deal to any extent with spectroscopic methods. The predominant impression derived from reading the book is that this must have been an outstandingly successful conference, with contributions of a uniformly high standard.

The cost of this book is undoubtedly high; it will probably only be acquired by Raman devotees. Nevertheless, the Editor and Publisher have done a great service, and those who were unable for any reason to attend the symposium will undoubtedly have considered it cheap at the price to be able to consult, only 10 months after the event, the original material that was presented.

D. M. W. ANDERSON

DAS ARBEITEN MIT IONENSELEKTIVEN ELEKTRODEN. EINE EINFÜHRUNG. By KARL CAMMANN. *Anleitungen für die chemische Laboratoriumspraxis, Band XIII*. Pp. xii + 226. Berlin, Heidelberg and New York: Springer-Verlag. 1973. Price DM56; $23.

This book, the thirteenth in the series of laboratory guides, is both attractive and systematic in its presentation. Following a brief introduction, the first quarter of the book is made up of two chapters devoted, respectively, to the principles of potentiometry and the measurement of electrode potential. In these, the reader is immediately made aware of the subject both by the slant of the treatment and by the sections concerned with the materials and selectivity of ion-selective electrodes.

The main business of the book is covered by the chapters on the various classes of ion-selective electrodes, measuring techniques, the techniques used in analysis, and areas of applications of ion-selective electrodes. All classes of ion-selective electrodes are covered, with discussions on construction principles, characteristics, operating principles and calibration, with useful tables on the main characteristics and suppliers of electrodes within each category. While certain emphases in the discussion of electrode types could have been better placed in order to avoid misconceptions, the section on gas-sensitive electrodes is a useful introduction to this intriguing elaboration of ion-selective electrodes.

Equivalent circuits and measuring instruments form the basis of the chapter on measuring techniques and, naturally, some of these aspects carry over to the chapter on analysis techniques. The standard techniques in the use of ion-selective electrodes are discussed, including known addition and Gran's methods. Additionally, some detailed experimental instructions are given, as in the determination of sodium and potassium in blood serum, but these, it must be emphasised, are limited in number.

The biomedical field and continuous industrial and environmental analysis are the themes of the final chapter, which is followed by a short discrete discussion on the way ahead for ion-selective

electrodes. In addition to data tables, the Appendix includes a discussion on activity, including references to the recent observations of Bates and Durst at the IUPAC-sponsored symposium held at UWIST, Cardiff, in April 1973. These, and the other two references to this symposium, are an indication of the speed with which the publishers have produced this book. Perhaps such despatch accounts for the high price which, together with the availability of so much information on ion-selective electrodes in English, is detrimental to giving the book a wide market.

J. D. R. THOMAS

METHODEN DER ORGANISCHEN ELEMENTAR- UND SPURENANALYSE. By F. EHRENBERGER and S. GORBACH. Pp. xvi + 452. Weinheim/Bergstrasse: Verlag Chemie. 1973. Price DM 129.

Fifteen years have elapsed since the last edition of "Pregl and Roth" (the standard text in the German language) appeared, and it is clearly time that a new edition, or an entirely new text dealing with more recent developments, should replace it. The first text-book on organic microanalysis, written by Pregl, ran to a number of editions. At least three English translations of this book have been published and, in addition, at least five independent English texts have been written by British or North American authors. Virtually none of the later books has had the opportunity to describe some of the notable advances that have taken place over the last decade, or even to include accounts of older methods that have since been well tested and tried under many different conditions. Accordingly, a comprehensive volume on elemental organic microanalysis would be expected to provide not only the working details of newer methods that have become well established, but also to provide a well balanced picture and a critical and selective assessment of investigations over the last quarter of a century. The present volume falls somewhat short of these expectations.

A generation ago it was not uncommon to find European authors complaining that their colleagues from another continent often appeared to be ignorant of earlier literature, and generally ascribed the pioneer work on any particular subject to the last authors who wrote about it. It was not a view to which I myself subscribed, except in a few individual cases, but the myth has certainly become fact in recent years in continents other than the one originally blamed. There are too many instances where the literature research has been carried out shoddily and important contributions have been omitted. This may well be due to the present-day chemist being over-pampered by the services that are available to him. In former times, there were few review articles available and one almost always had to go to the original literature for complete information. Nowadays, thanks to the proliferation of review articles, these are too often relied on for all background information, which is how so many of the myths and legends arise. Although the present volume provides comparatively few such errors, these may well have originated in this way.

The book confines itself to elemental analysis; this is a wise choice, because the conventional microanalytical laboratory rarely undertakes functional group analysis nowadays, owing to the development of instrumental techniques. Methods for all the conventional elements are described in detail. There is a very scanty section on microgram analysis, but the details are so meagre that it would have been best left out. Finally, there are chapters on statistics and on balances and weighing.

There is a reasonably comprehensive description of methods for the determination of carbon and hydrogen, including the most important automatic methods. Curiously enough, although the origin of the empty tube method is correctly ascribed, the system the authors recommend is one published many years after the original descriptions and which the reviewer had never seen before. This is remarkable, as the empty tube design has been a British Standard specification for about 20 years and, until the advent of automatic analysers, was the most widely used method in Britain. Paradoxically, the equivalent apparatus used for halogens and sulphur, which was not so widely used after the revival of the oxygen-flask method, is described in detail.

The chance was lost to place in a text for the first time the historic development of the oxygen-flask method; instead, it is covered simply by references. There was also the chance to give Mikl and Pech full credit for their contribution. To them, more than anybody else in the post-war period, must go the credit for resuscitating the oxygen-flask method. Theirs was a semimicro method using a 500-ml flask; Schöniger's contribution was to reduce the semimicro method to the micro scale. Mikl and Pech seem to be doomed to be deprived of their rightful place, which can only be ascribed to the reasons given in the second paragraph.

On the favourable side, one can say that the methods are described in detail and anyone who uses this book will have little difficulty in following the instructions. It might be thought that

too many methods have been provided, but this criticism is only justifiable when methods are merely alternatives to each other. No method is capable of analysing every compound; even the automatic methods are not universally applicable for the determination of carbon and hydrogen and the Burger - Zimmermann method is the only dependable method for determining sulphur in most organometallic compounds; availability of apparatus may also affect the method of choice. Accordingly, one can say that a fair choice of methods has been provided.

It can be said that this book puts more emphasis on methods developed on the Continent of Europe rather than in the UK and the USA, which is, after all, to be expected; it must not be forgotten that organic microanalysis originated in Austria. The general account is thus probably representative of preferred European practice. After having said that, I do not think this text is as good as the best one of those available in English, even though the latter needs to be up-dated; hence there would be little advantage in preparing a translation of this volume.

Pre-war, one could have bought half a micro-balance for the cost of this book.

R. BELCHER

AN INTRODUCTION TO SEPARATION SCIENCE. By BARRY L. KARGER, LLOYD R. SNYDER and CSABA HORVATH. Pp. xxii + 586. New York, London, Sydney and Toronto: John Wiley & Sons. 1973. Price £9·75.

The authors set out to present, in three parts, a unified view of the numerous separation techniques that are currently available. They are assisted by nine specialists, presenting salient features of particular techniques.

Part I, "Fundamentals," considers in turn separation equilibria, diffusion and mass transport, operational aspects of separation, chromatography and finally characteristics of individual separation methods. Separation equilibria are discussed thermodynamically and on a molecular basis; this latter aspect is often neglected but is a model of clarity of exposition in this section.

Part II, "Methods based on Phase and Distribution Equilibria," contains chapters on distillation (R. H. McCormick), gas - liquid chromatography, solvent extraction (H. Freiser), liquid - liquid chromatography, crystallisation (W. R. Wilcox), ion-exchange separation processes (H. L. Rothbart), liquid - solid adsorption chromatography, various other interfacial processes and finally exclusion processes (J. Y. Chuang and J. F. Johnson).

Part III, "Other Separation Methods," covers barrier separation processes (R. A. Cross and H. Strathmann), electrophoresis (M. Bier), miscellaneous methods including ultracentrifugation, particle size, electromagnetic separation, thermal diffusion, use of enzymes and finally a chapter on multi-step separation schemes for complex samples.

The chapters on individual techniques cannot be compared to monographs, as ten to twenty pages are not sufficient to develop detailed discussions and tabulate exhaustive lists of examples. As introductions, placing techniques in perspective and providing key references, most chapters are considered to be useful. The last chapter, on multi-step separations, outlines the stages in the determination of aldosterone in human urine, the isolation of 13-methylhentriacontane from corn earworm faeces, analysis of individual skin lipids and the separation and analysis of the oxygen- and nitrogen-containing compounds, or both, in petroleum. These schemes illustrate many of the techniques outlined earlier in the book and are a realistic and fitting conclusion to it.

The presentation and diagrams are of a high quality and few typographical errors were noted.

The book achieves its aims and will repay study by both academic and industrial analytical chemists and others such as biochemists with separation problems or interests.

D. THORBURN BURNS

Errata

NOVEMBER (1973) ISSUE, p. 825, line 14 of text: *for "Preparation of sample"* read *"Preparation of sample mixture."* Line 19 of text: *for* "sample size" *read* "injection volume."

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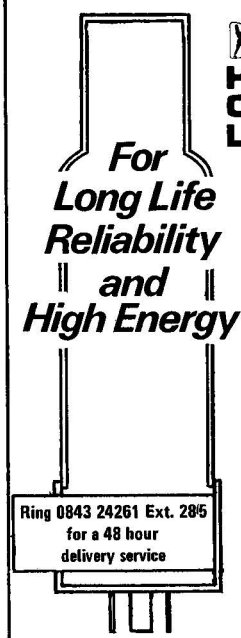

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Colorimetric Determination of Small Amounts of C_8 to C_{10} Alcohols in Their Phthalate Esters

Methods were examined for the determination of small amounts of C_8 to C_{10} alcohols in their phthalate esters involving the use of vanadium 8-hydroxyquinolinate in benzene or toluene and 3,5-dinitrobenzoyl chloride in pyridine as colorimetric reagents. Two modified procedures are presented that will reliably determine free alcohols in the 0·01 to 0·4 per cent. *m/m* range.

S. HARRISON, H. HINCHCLIFFE and G. L. WOODROFFE

Research and Development Department, Imperial Chemical Industries Limited, Petrochemicals Division, Billingham, Teesside, TS23 1JB.

Analyst, 1974, **99**, 491–497.

Determination of Primary and Secondary Amines Alone and in Mixtures with Tertiary Amines

An iodatometric method has been developed for the determination of primary and secondary amines that is based on their quantitative reaction with phenyl isothiocyanate in dimethylformamide to form substituted thioureas. These are titrated with potassium iodate in an acidic medium at room temperature. The end-point is detected visually by the yellow colour imparted to the solution by the first drop of the iodate solution in excess, and potentiometrically by using a bright platinum-wire indicator electrode and a saturated calomel reference electrode. Methods have also been developed for the determination of primary (or secondary) amines and tertiary amines in the presence of each other. An excess of phenyl isothiocyanate, added to the mixture in solution in dimethylformamide, converts the primary (or secondary) amines into the corresponding di- (or tri-) substituted thioureas, whereas the tertiary amines are left unreacted. The conductimetric titration of tertiary amines with trichloroacetic acid, followed by the iodatometric titration of thioureas formed, enables the mixture to be analysed for both components. The methods described are simple, accurate and reliable.

BALBIR CHAND VERMA and SWATANTAR KUMAR

Department of Chemistry, Punjabi University, Patiala, India.

Analyst, 1974, **99**, 498–502.

A Technique for the Determination of Trace Anions by the Combination of a Potentiometric Sensor and Liquid Chromatography, with Particular Reference to the Determination of Halides

A technique for the determination of trace amounts of halides in the presence of other ions is described. The species are separated by means of liquid chromatography and detected potentiometrically by a silver - silver chloride micro-electrode. The technique readily lends itself to automation and an apparatus for rapid, repetitive analyses has been designed. By careful choice of the eluting agent and stationary phase it is possible to achieve a variety of separations, e.g., the determination of chloride in the presence of excess of sulphide, the separation and determination of nanogram amounts of chloride, bromide and iodide in mixed halide solutions and the determination of chloride in boiler waters.

In addition, conditions for the extension of the technique to the separation and determination of other anions are proposed.

M. C. FRANKS and D. L. PULLEN

BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex, TW16 7LN.

Analyst, 1974, **99**, 503–514.

A Method for the Determination of Total Sulphur in Silicate Rocks

After oxidising sulphides to sulphates with a mixture of sodium chlorate and hydrochloric acid, the sample is refluxed with a reducing mixture of sodium iodide, red phosphorus, hypophosphorous acid, orthophosphoric acid and propionic acid. The hydrogen sulphide generated is absorbed in potassium hydroxide solution and titrated against 2-(hydroxymercuri)benzoic acid solution with dithizone as indicator. The approximate range covered is 5 to 2000 mg kg^{-1} of sulphur in the rock.

J. M. MURPHY and G. A. SERGEANT

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

Analyst, 1974, **99**, 515–518.

A Titrimetric Method for the Determination of Sulphate in Fertilisers

A method is described for the determination of sulphate in fertilisers in which the sulphate is precipitated with barium chloride from an acidified EDTA solution. The precipitate is filtered off by using membrane filters and is dissolved in ammoniacal EDTA; the excess of EDTA is then titrated against a solution of magnesium ions with Eriochrome black T as the indicator. No interference is encountered from iron, aluminium, fluoride or phosphate ions.

A. D. CAMPBELL, D. P. HUBBARD and N. H. TIOH

Department of Chemistry, University of Otago, Box 56, Dunedin, New Zealand.

Analyst, 1974, **99**, 519–522.

Loss of Zinc and Cobalt During Dry Ashing of Biological Material

Loss of zinc and cobalt during dry ashing of marine mussels (*Mytilus edulis*) and brown seaweed (*Fucus spiralis*) has been studied by using material labelled by exposure of living organisms to sea water spiked with zinc-65 or cobalt-60. Even after ashing in porcelain crucibles at temperatures of up to 1000 °C, no significant loss of zinc or cobalt by volatilisation was observed. After ashing at 450 and 550 °C, both radionuclides could be removed quantitatively from the crucibles by leaching with hydrochloric acid. Adsorption on the crucible after ashing at 1000 °C was measured only for cobalt-60. A significant proportion of the cobalt tracer could not be removed from the crucibles by treatment of the latter with acid. From these results it is concluded that dry ashing is a reliable method of sample destruction for the determination of zinc and cobalt in *M. edulis* and *F. spiralis*.

J. G. van RAAPHORST, A. W. van WEERS and H. M. HAREMAKER

Reactor Centrum Nederland, Petten, The Netherlands.

Analyst, 1974, **99**, 523–527.

Determination of Copper(I) with *N*-Bromosuccinimide

It has been found that *N*-bromosuccinimide readily and quantitatively oxidises aqueous solutions of copper(I) at room temperature and in the presence of dilute hydrochloric acid, the oxidising agent being irreversibly reduced to succinimide.

A procedure is suggested for the determination of copper(I) by titration with standard *N*-bromosuccinimide solution; the results obtained were found to be equally precise but more accurate than those given by the permanganate and complexometric methods.

Copper(II), after preliminary reduction, can also be determined by the suggested procedure, whether alone or admixed with copper(I).

A. ABOU EL KHEIR, M. AYAD and M. M. AMER

Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Analyst, 1974, **99**, 528–532.

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CONTENTS

ORIGINAL PAPERS

	Page
The Determination of Trace Amounts of Lead in Steel by Anodic Stripping Voltammetry—B. Metters and B. G. Cooksey	457
A Modification to the Extraction-Atomic-absorption Method for the Determination of Antimony, Bismuth, Lead and Tin—K. Thornton and Keith E. Burke	469
Spectrophotometric Determination of Magnesium in Tobacco Leaves with Eriochrome Black B—G. Slegers and A. Claeys	471
The Spectrophotometric Determination of Ampicillin and Cloxacillin in Combined Injections—A. G. Davidson and J. B. Stenlake	476
Colorimetric Determination of Piperazine in Pharmaceutical Formulations —Yehia M. Dessouky and Saad A. Ismaiel	482
Colorimetric Determination of Antazoline in Some Pharmaceutical Preparations with Sodium Nitrite—M. M. Amer, M. S. Tawakkol and S. A. Ismaiel	487
Colorimetric Determination of Small Amounts of C_8 to C_{10} Alcohols in Their Phthalate Esters—S. Harrison, H. Hinchcliffe and G. L. Woodroffe	491
Determination of Primary and Secondary Amines Alone and in Mixtures with Tertiary Amines—Balbir Chand Verma and Swatantar Kumar	498
A Technique for the Determination of Trace Anions by the Combination of a Potentiometric Sensor and Liquid Chromatography, with Particular Reference to the Determination of Halides—M. C. Franks and D. L. Pullen	503
A Method for the Determination of Total Sulphur in Silicate Rocks— J. M. Murphy and G. A. Sergeant	515
A Titrimetric Method for the Determination of Sulphate in Fertilisers— A. D. Campbell, D. P. Hubbard and N. H. Tioh	519
Loss of Zinc and Cobalt During Dry Ashing of Biological Material—J. G. van Raaphorst, A. W. van Weers and H. M. Haremaker	523
Determination of Copper(I) with *N*-Bromosuccinimide—A. Abou El Kheir, M. Ayad and M. M. Amer	528
Book Reviews	533
Errata	536
Summaries of Papers in this Issue	iii, v, viii, x

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