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THE ANALYST

NOTICE TO SUBSCRIBERS AND AUTHORS

Delays in Publication

WHILE the present emergency regulations in the UK are in force, the Society's printers are experiencing difficulties in keeping to schedules as a result of short-time working, and although every effort is being made to maintain production, it is inevitable that delays will occur.

Ways of alleviating this situation include setting fewer papers in type, which reduces the load on the compositors, and printing smaller issues, which reduces the number of machinings required to print the final journal. Accordingly, this issue of ***The Analyst*** contains only 64 pages and subsequent issues will contain 48 pages until full production can be resumed.

It is regretted that these steps have become necessary and that publication is being delayed. In the meantime, available papers are being processed in the Editorial Office and will be ready for use when circumstances permit.

The Society apologises to both subscribers and authors; it is intended eventually to publish the backlog in larger issues of ***The Analyst*** and then to revert to the normal publication schedule as soon as possible.

Summaries of Papers in this Issue

Ionic Polymerisation as a Means of End-point Indication in Non-aqueous Thermometric Titrimetry

Part V. The Iodimetric Determination of Organic Bases, Hydrazine Derivatives and Water

A thermometric titration method has been evaluated in which organic bases, hydrazines, phosphines and quaternary ammonium halides, and also water, have been titrated with iodine in non-aqueous solutions containing alkyl vinyl ethers. The latter polymerise with the excess of iodine evolving heat, which marks the end-point.

The ratio of the reactants in titrations of most of the amines examined, namely 3·6 to 4·6 atoms or 1·8 to 2·3 molecules of iodine to 1 molecule of amine, depending on the amine, is favourable to the titration. With hydrazine derivatives, the ratio ranges from 4·2 to less than 1 atom of iodine to 1 molecule of the hydrazine, depending on the hydrazine derivative.

Water can be titrated with iodine in the presence of alkyl vinyl ethers, about thirteen molecules of water consuming one atom of iodine.

The end-point in titrations of most of the compounds examined is marked by a sharp inflection in the titration graph when an automatic procedure is used. Precisions are usually better than 1 per cent. with 0·05 M and 2 per cent. with 0·01 M titrant solutions.

Sample sizes down to about 0·0005 mmol, depending on the iodine consumed in the reaction, can be determined with 0·01 M titrant solution. Calibration graphs show that, except in the titration of water, the volume of titrant and amount of sample are linearly related in the range 0 to 1 ml of titrant. The curvatures of calibration graphs for water depend on the rates of addition of iodine to the sample; linearity can almost be achieved at an appropriate titration rate.

It is suggested that the stoicheiometry, *i.e.*, the iodine consumed per molecule of sample, is a quantitative measurement of the basic properties of the compounds investigated. The different stoicheiometries for different compounds make the iodimetric method useful for the selective determination of the constituents of binary mixtures of bases and hydrazine derivatives, but unsuitable for the determination of the total basic or hydrazine function in more complex mixtures.

E. J. GREENHOW and L. E. SPENCER

Department of Chemistry, Chelsea College, University of London, Manresa Road, London, S.W.3.

Analyst, 1974, **99**, 82–92.

Problems in the Determination of Carbon in Steel by a Precision Coulometric Method

An apparatus for the coulometric determination of carbon in steel with a precision approaching 1 part in 1000 at the 1 per cent. level has been constructed. The measures needed to overcome sources of error in gas absorption, pH measurement, control and coulometric measurement are described. The design avoids the use of a gas proportionating pump and can be used with a 0·2-g sample containing up to 2 per cent. of carbon, and proportionately larger amounts up to 2 g for lower carbon contents. Results on a series of standard steels are given.

J. D. HOBSON and H. LEIGH

Dunford Hadfields Ltd., East Hecla Works, Sheffield, S9 1TZ.

Analyst, 1974, **99**, 93–107.

THE ANALYST

Editorial

Hazardous Chemicals as Reagents

We live in an age of increasing anxiety about "the quality of life," and its impairment by technological advances. Fears are expressed about pollution of the atmosphere, pesticide residues in food, the dumping of toxic wastes on land or into rivers and oceans, the safety of industrial processes and transport systems, the carnage and nuisance factors arising from high traffic densities, noise levels in cities, and so on. Legislation relevant to various such factors has been enacted or is under consideration in many countries.

The tide of opinion in this direction, and the consequent legislation, are of considerable import for the analyst. It is he who has to measure pollution levels in the atmosphere, the amounts of toxic materials in industrial effluents and in river and other waters, and the concentrations of toxic vapours to which personnel may be exposed in industrial processes. In these and many similar ways the analyst has a vital rôle to play in seeking to contain within reasonable bounds the many hazards and nuisances than can arise in present-day life. The analytical work required in such contexts can call for expertise of a high order, particularly when pollutants or contaminants need to be measured at very low levels of concentration.

Against this background it is similarly important for the analyst to ensure that his own house is in order. Clearly the analyst himself should seek to avoid unnecessary hazards in his work either by eliminating the use of dangerous substances or procedures or, when this is impracticable, by ensuring that the recognised safety precautions are taken. A particular problem for the analyst can arise when a long established and widely used reagent is belatedly found to have hazardous properties; examples are the amines benzidine and *o*-tolidine, now known to be carcinogenic.

This type of problem extends further, into the realms of publication of scientific papers in primary journals. Thus, is it ethical to accept for publication a paper advocating the use of a reagent known to be a hazardous substance, particularly when alternative reagents of a safer nature may be applicable?

This dilemma has recently been faced by the Executive Committee for *The Analyst*, in connection with a paper in this issue (p. 128) concerned with the use of *o*-tolidine. The decision reached by the Committee was that it had no right to censor or prevent the publication of any scientific paper solely on the grounds of its advocating the use of a reagent known to be a hazardous substance. However, the Committee has deemed it essential that any such paper published in *The Analyst* should make clear the hazardous nature of the reagent used, and should include information on or references to the appropriate safety precautions to be adopted.

This policy has been put into effect in relation to the particular paper on p. 128 of this issue, and will be applied to any future paper in *The Analyst* involving substances or procedures recognised to be abnormally hazardous.

H. J. Cluley
Chairman, Analyst Executive Committee

Analyst, February, 1974, Vol. 99, *pp.* 82–92

Ionic Polymerisation as a Means of End-point Indication in Non-aqueous Thermometric Titrimetry

Part V.* The Iodimetric Determination of Organic Bases, Hydrazine Derivatives and Water

BY E. J. GREENHOW AND L. E. SPENCER

(*Department of Chemistry, Chelsea College, University of London, Manresa Road, London, S.W.3*)

A thermometric titration method has been evaluated in which organic bases, hydrazines, phosphines and quaternary ammonium halides, and also water, have been titrated with iodine in non-aqueous solutions containing alkyl vinyl ethers. The latter polymerise with the excess of iodine evolving heat, which marks the end-point.

The ratio of the reactants in titrations of most of the amines examined, namely 3·6 to 4·6 atoms or 1·8 to 2·3 molecules of iodine to 1 molecule of amine, depending on the amine, is favourable to the titration. With hydrazine derivatives, the ratio ranges from 4·2 to less than 1 atom of iodine to 1 molecule of the hydrazine, depending on the hydrazine derivative.

Water can be titrated with iodine in the presence of alkyl vinyl ethers, about thirteen molecules of water consuming one atom of iodine.

The end-point in titrations of most of the compounds examined is marked by a sharp inflection in the titration graph when an automatic procedure is used. Precisions are usually better than 1 per cent. with 0·05 M and 2 per cent. with 0·01 M titrant solutions.

Sample sizes down to about 0·0005 mmol, depending on the iodine consumed in the reaction, can be determined with 0·01 M titrant solution. Calibration graphs show that, except in the titration of water, the volume of titrant and amount of sample are linearly related in the range 0 to 1 ml of titrant. The curvatures of calibration graphs for water depend on the rates of addition of iodine to the sample; linearity can almost be achieved at an appropriate titration rate.

It is suggested that the stoicheiometry, *i.e.*, the iodine consumed per molecule of sample, is a quantitative measurement of the basic properties of the compounds investigated. The different stoicheiometries for different compounds make the iodimetric method useful for the selective determination of the constituents of binary mixtures of bases and hydrazine derivatives, but unsuitable for the determination of the total basic or hydrazine function in more complex mixtures.

THE determination of organic bases by iodimetric titration has not previously been considered, although reactions between pyridine and iodine at ambient temperature were reported as early as 1895.[1] Pyridinium bromide perbromide ($C_5H_5N.HBr.Br_2$) and trimethylphenylammonium tribromide are available as laboratory reagents but the simple amine - halogen addition compounds appear to have received little attention. Indeed, in a recent edition of one of the standard works on organic chemistry,[2] it is noted that the reaction between halogens and primary and secondary amines "does not give useful products."

The titration of organic hydrazine derivatives with iodine, bromine or binary interhalogen compounds of iodine, bromine and chlorine, is an established assay procedure.[3] The oxidation reaction is usually, but not always, accompanied by the evolution of nitrogen. With hydrazides, four atoms, *i.e.*, two molecules, of iodine are normally consumed by one molecule of hydrazide, but it has been pointed out[3] that reactions of halogens with hydrazine derivatives in general may be complicated by there being more than one possible route and, consequently, variations in the stoicheiometry. It has been stressed that strict adherence to experimental conditions is necessary in order to obtain reproducible results.

* For Part IV of this series, see *Analyst*, 1973, **98**, 485.

Iodimetry, in the form of the Karl Fischer reaction, is the most important chemical method for the determination of water in organic solvents. In this reaction, two atoms of iodine are, in theory, consumed by one molecule of water.

Siggia[4] has shown that iodine reacts with vinyl ethers in the presence of an alcohol according to the following equation:

$$ROCH{=}CH_2 + I_2 + R'OH \rightarrow ROCH(OR')CH_2I + HI$$

It would seem likely, therefore, that water should undergo a similar reaction in which two atoms of iodine would correspond to one molecule of water. The reaction product in this instance, $ROCH(OH)CH_2I$, could conceivably react with further iodine and vinyl ether, raising the reaction ratio to four atoms of iodine to one molecule of water.

The use of catalytic thermometric titration for the determination of organic bases is described in Part I.[5] More recently, a catalytic thermometric procedure has been reported[6] for the determination of hydrazine derivatives and xanthates and, less effectively, for thiols. In this procedure iodine was used as the titrant and as a catalyst for the cationic polymerisation process that indicates the end-point, and ethyl vinyl ether was used as the monomer. A detailed investigation of this procedure, which forms the basis of the present paper, has shown that in non-aqueous solution iodine can be used as a titrant not only for certain oxidisable compounds, such as hydrazine derivatives, but also for organic bases and water.

A number of organic solvents for the titrant (iodine) and titrands have been assessed in an effort to achieve the optimum determination conditions. In addition, iodine bromide and iodine chloride have been examined as alternative titrants and some alkyl vinyl ethers other than ethyl vinyl ether have been examined as alternative monomers.

Experimental

Reagents—

Laboratory-reagent grade dimethylformamide, toluene, pyridine, *NN*-dimethylacetamide, 1,2-dichloroethane, tetrahydrothiophene 1,1-dioxide (sulpholane) and dimethyl sulphoxide were dried over molecular sieve 4A before use.

Iodine and acetic acid (both AnalaR grade) were used as received.

Ethyl vinyl ether, n-butyl vinyl ether, 2-chloroethyl vinyl ether, isobutyl vinyl ether, other organic bases, benzoic acid, iodine bromide, iodine chloride and iodine trichloride were laboratory-reagent grade materials and were used without further purification. Divinyl ether was extracted with distilled water and dried over alumina before use.

Benzenesulphonohydrazide, 4,4′-oxybis(benzenesulphonohydrazide) and 2-hydroxyethylhydrazine were gifts from Fisons Limited, Agrochemicals Division. Other hydrazine derivatives were laboratory-reagent grade materials.

Solutions of iodine, 0·05 and 0·01 M in organic solvents, were standardised by adding to 20 ml of the solutions 50 ml of water, 1 g of potassium iodide and 3 ml of 1 M sulphuric acid, and titrating with 0·1 and 0·01 M sodium thiosulphate solutions, respectively, with starch as indicator.

Solutions of iodine chloride, iodine trichloride and iodine bromide in dimethylformamide, all 0·05 M, were standardised by the method used for 0·05 M iodine solution.

Apparatus—

For thermometric titration—Use the automatic apparatus described in Part III[7] with an 8-ml titration flask.

For gasometric determinations—Use a 50-ml gas burette, connected to a 50-ml, magnetically stirred reaction vessel with a side-arm that is fitted with a serum cap. Details of the apparatus were given by Dixon.[8]

Procedure—

Thermometric titration—Prepare a solution of the sample in dimethylformamide or in another appropriate solvent. The concentration will depend on the stoicheiometry of the reaction with iodine and on the titrant concentration; thus 1 ml of the solution should contain about 0·1 mequiv of the sample compound when the 0·05 M titrant is used and about 0·02 mequiv with 0·01 M titrant.

Transfer by pipette 1 ml of the sample solution into the reaction flask, add 2 ml of ethyl vinyl ether, stir the solution, then add titrant at the rate of about 0·1 ml min^{-1} from the motor-driven syringe. The titrant volume at the end-point is taken to be the volume corresponding to the point of inflection in the titration graph. When this inflection is indistinct, the end-point is taken to be the point where the tangent to the main heat rise leaves the graph at its lower temperature end.

Carry out a blank titration by using an equal volume of the same batch of solvent, with the same water content, as that used for the sample titration.

The chart recorder of the automatic titration apparatus is conveniently operated at a chart speed of 600 mm h^{-1} and in the range 0 to 100 mV with 0·05 M titrant and 0 to 50 mV with 0·01 M titrant.

Gasometric determinations—Transfer by pipette 5 ml of a 0·8 M solution of iodine in dimethylformamide into the reaction vessel, sweep out the air in the vessel with dry nitrogen and connect the vessel to the gas burette. Stir the iodine solution and add, by injection from a syringe through the serum cap, 1-ml aliquots of a 0·18 M solution of the hydrazine derivative in dimethylformamide at 5-minute intervals, reading the gas burette immediately before each injection. Correct the observed increases in volume to volumes at S.T.P. and subtract from these values the volume (1 ml) of the sample solution injected.

For the reverse of the above procedure, *i.e.*, addition of aliquots of iodine to an excess of the hydrazide, carry out the above operations, but with 4 mmol of hydrazide dissolved in 10 ml of dimethylformamide in the reaction flask and with 1-ml aliquots of 0·5 M iodine.

Results and discussion

A further investigation of the catalytic thermometric titration of isonicotinoylhydrazine with iodine in dimethylformamide solution, reported briefly in an earlier paper,[6] has revealed that: (*a*), addition of an equimolar amount of acetic or benzoic acid to the hydrazide prior to titration has a negligible effect on the titration value; (*b*), addition of small amounts, *e.g.*, 1 per cent., of pyridine or water to the hydrazide prior to titration significantly increases the titration value but does not reduce the sharpness of the end-point; and (*c*), benzoylhydrazine requires much less titrant (1·4 atoms of iodine per molecule) than does isonicotinoylhydrazine (4·2 atoms of iodine per molecule).

Observation (*b*) above indicates that both pyridine and water are titrated iodimetrically by the catalytic thermometric procedure.

By using the procedure, a number of pyridine derivatives, primary, secondary and tertiary aliphatic and alicyclic amines, quaternary ammonium halides, organophosphorus derivatives and heterocyclic compounds containing two nitrogen atoms, have been determined. In Table I the compounds are listed and the reactivities in terms of the number of atoms of iodine combining with one molecule of the compound are given. Typical titration graphs are shown in Figs. 1 and 6.

It can be seen that, if the inflection point of the iodimetric titration is taken as the basis for the calculation, pyridine combines with 4·1 atoms of iodine in dimethylformamide solution. This result agrees well with the formula $C_5H_5N.2I_2$ obtained by Prescott and Trowbridge[1] for the crystalline adduct of iodine with pyridine.

Most of the aliphatic amines and pyridine derivatives required from 3·6 to 4·6 atoms of iodine for each molecule in the titration. It is interesting to note that the secondary amines are less reactive towards iodine than are the tertiary amines, including the pyridine derivatives, although the dissociation constants of aliphatic bases show the secondary amines to be stronger bases than the tertiary amines in aqueous solution.

Aniline and the alkylanilines, as might be expected from their weakly basic character, show much lower reactivity towards iodine. Substitution of electron-withdrawing groups on the aniline and pyridine rings causes a reduction in the reactivity towards iodine. Thus, *p*-nitroaniline does not combine with iodine and 2,6-pyridinedicarboxylic acid shows very low reactivity. Diphenylamine and triphenylamine are unreactive for the same reason.

In contrast with triphenylamine, triphenylphosphine combines with iodine in the ratio of about 1 atom of iodine to 1 molecule of triphenylphosphine but the inflection at the end-point is not sharp (Fig. 1).

The *N*-oxides of pyridine and 3-picoline were found to react with iodine in an approximate ratio of 2 atoms of iodine to 1 molecule of the *N*-oxide but, again, there was no distinct

TABLE I

ORGANIC BASES AND QUATERNARY AMMONIUM HALIDES TITRATED WITH 0·05 M IODINE IN DIMETHYLFORMAMIDE

Conditions: 0·025 mmol of base or halide in 1 ml of dimethylformamide added to 2 ml of ethyl vinyl ether and titrated by the thermometric procedure

Aliphatic and alicyclic amines—
n-Butylamine (3·6); benzylamine (3·7); 1,2-diaminoethane (6·75); morpholine (3·6); piperidine (3·6); triethylamine (4·2); tris(hydroxymethyl)methylamine (4·3); 2-*NN*-diethylaminopropionitrile (4·4); *N*-methylmorpholine (4·6); and *N*-ethylpiperidine (4·4)

Pyridine derivatives—
Pyridine (4·1); α-picoline (3·8); 2,6-lutidine (4·0); 2,6-pyridinedicarboxylic acid (0·09); pyridine *N*-oxide (2·2); 3-picoline *N*-oxide (2·2); quinoline (4·1); 4-methylquinoline (4·3); 8-hydroxyquinoline (2·1); and 2-hydroxyquinoline (0)

Aniline derivatives—
Aniline (1·3); *o*-toluidine (1·5); *p*-toluidine (1·8); *p*-nitroaniline (0); diphenylamine (0); and triphenylamine (0)

Heterocyclic nitrogen compounds—
Hexamethylenetetramine (5·0); phthalazine (3·5); quinoxaline (0); and benzimidazole (4·0).

Quaternary ammonium halides—
Tetra-n-butylammonium bromide (1·8); tetra-n-butylammonium iodide (1·5); cetyltrimethylammonium bromide (1·4); cetylpyridinium bromide (1·8); and benzyldimethylmyristylammonium chloride (2·4)

Phosphorus compounds—
Triphenylphosphine (1·1); and triphenylphosphine oxide (0)

Figures in parentheses following the name of the compound denote the number of iodine atoms combining with one molecule of the compound on the basis of the iodimetric titration.

inflection point in the titration graph and the end-points were difficult to establish (Fig. 1). Triphenylphosphine oxide did not react with iodine.

Quinoline and 4-methylquinoline are similar to pyridine and the methylpyridines in their reactivities towards iodine, but 8-hydroxyquinoline is far less reactive and 2-hydroxyquinoline shows no reaction. Presumably hydrogen bonding in the last two compounds inhibits or prevents formation of adducts with iodine.

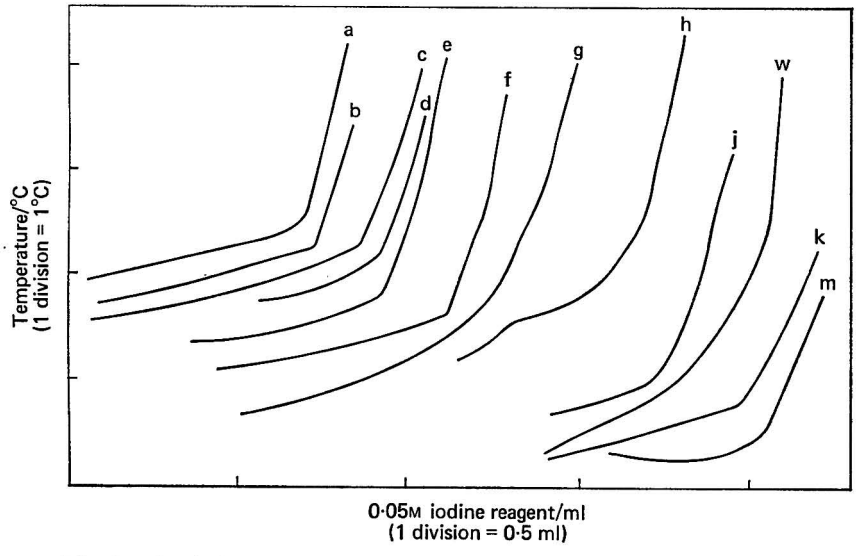


Fig. 1. Catalytic thermometric titration of organic bases, water and quaternary ammonium halides with 0·05 M iodine reagent. Compounds/mg: a, n-butylamine, 0·84; b, tris(hydroxymethyl)methylamine, 1·2; c, pyridine, 1·3; d, hexamethylenetetramine, 1·6; e, morpholine, 1·89; f, 8-hydroxyquinoline, 2·9; g, pyridine *N*-oxide, 2·3; h, triphenylphosphine, 7·4; j, cetyltrimethylammonium bromide, 4·0; k, tetra-n-butylammonium iodide, 9·3; m, benzyldimethylmyristylammonium chloride, 5·0; and w, water, 10·0

A number of heterocyclic compounds containing more than one ring nitrogen atom have been titrated. Hexamethylenetetramine combines with only five atoms of iodine instead of the possible sixteen, calculated on the basis of four atoms of iodine for each nitrogen atom. Quinoxaline proved to be unreactive but phthalazine, an isomer of quinoxaline, combined with 3·5 atoms of iodine per molecule. Presumably the more remote nitrogen atoms in phthalazine are less affected by the benzene ring. Benzimidazole, with one of its two nitrogen atoms unconjugated to the benzene ring, is even more reactive than phthalazine.

Quaternary ammonium halides, including alkylpyridinium halides, can be titrated by means of this iodimetric method. With the compounds examined it was found that from 1·4 to 2·4 atoms of iodine combined with one molecule of the halide. There appears to be a tendency to form trihalides, which are known to be stable systems, and for the halide ion to influence the reactivity.

The possibility of water reacting with iodine in the presence of vinyl ethers was discussed in the introduction. It has been confirmed that water can be titrated in solution in dimethylformamide and that a sharp end-point is obtained (Figs. 1 and 6). There is some indication that the presence of water increases the sharpness of the end-point in the titration of other compounds. In the titration of water, the consumption of the iodine titrant at the indicated end-point is dependent on the rate of addition of the titrant, as shown in Fig. 2. It can be seen that there is an almost linear relationship between the water content of the sample and the titrant required when titrant is added at a rate of 0·06 ml min^{-1}. This rate would, therefore, be the recommended rate of addition of titrant in the determination of water and, at this rate of addition, about one atom of iodine is consumed by thirteen molecules of water. Clearly, the method is not highly sensitive for the determination of water because 23·4 mg of water would require only 1 ml of 0·05 M iodine solution on the basis of the above reaction ratio.

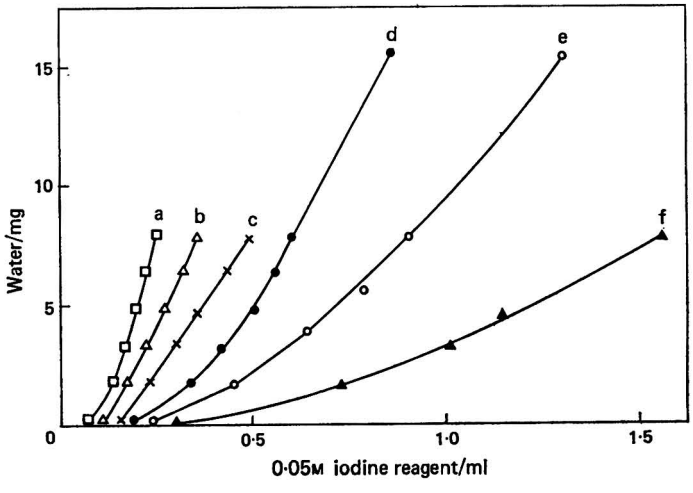


Fig. 2. Effect of the rate of addition of titrant on calibration graphs in the thermometric titration of water with 0·05 M iodine reagent. Rate of addition of titrant/ml min^{-1}: a, 0·02; b, 0·032; c, 0·06; d, 0·12; e, 0·20; and f, 0·60

The reaction of water, iodine and ethyl vinyl ether in dimethylformamide does not appear to be simple and, if it is similar to the reaction described by Siggia[4] for alcohols, obviously does not proceed to completion during the course of the titration. It is possible, however, that water is being titrated merely as a weak base.

As water is titrated, an allowance must be made when organic solvents containing trace amounts of water are used in the titration of bases, hydrazine derivatives, etc. A convenient procedure is to carry out a blank titration on an equal volume of the same solvent, taken

from the same batch as that used for dissolving the sample. With dimethylformamide dried over molecular sieve 4A, the blank titration with 0·05 M iodine is about 0·15 ml. It is important, of course, that the sample itself should be dry.

In addition to isonicotinoylhydrazine, a number of other hydrazine derivatives have been determined, including hydroxyalkyl-, dialkyl-, aryl- and diarylhydrazines, arylhydrazides, arylsulphonohydrazides and semicarbazides. In Table II, the reactivities of these compounds towards iodine are given in terms of iodine atoms per molecule.

TABLE II

HYDRAZINE DERIVATIVES TITRATED WITH 0·05 M IODINE IN DIMETHYLFORMAMIDE

Conditions: Sufficient compound to give a titration value of about 0·5 ml is dissolved in 1 ml of dimethylformamide, added to 2 ml of ethyl vinyl ether and titrated by the thermometric procedure

NN-Dimethylhydrazine (3·4); 2-hydroxyethylhydrazine (2·2); phenylhydrazine (1·0); 4-nitrophenylhydrazine (1·1); 2,4-dinitrophenylhydrazine (0·18); *NN*-diphenylhydrazine (0·86); benzoylhydrazine (1·4); isonicotinoylhydrazine (4·2); benzenesulphonohydrazide (0·33); 4,4′-oxybis(benzenesulphonohydrazide) (0·63); semicarbazide hydrochloride (1·9); and thiosemicarbazide (3·1)

Figures in parentheses following the name of the compound denote the number of iodine atoms combining with one molecule of the compound on the basis of the iodimetric titration.

The alkylhydrazines, *i.e.*, *NN*-dimethylhydrazine and 2-hydroxyethylhydrazine, were the most reactive of the hydrazine derivatives examined, if one excepts thiosemicarbazide and isonicotinoylhydrazine, which possess reactive groups other than the hydrazine group. Titration graphs for some of the hydrazine derivatives are shown in Fig. 3. It can be seen that in the titration of benzenesulphonohydrazide, 4,4′-oxybis(benzenesulphonohydrazide)

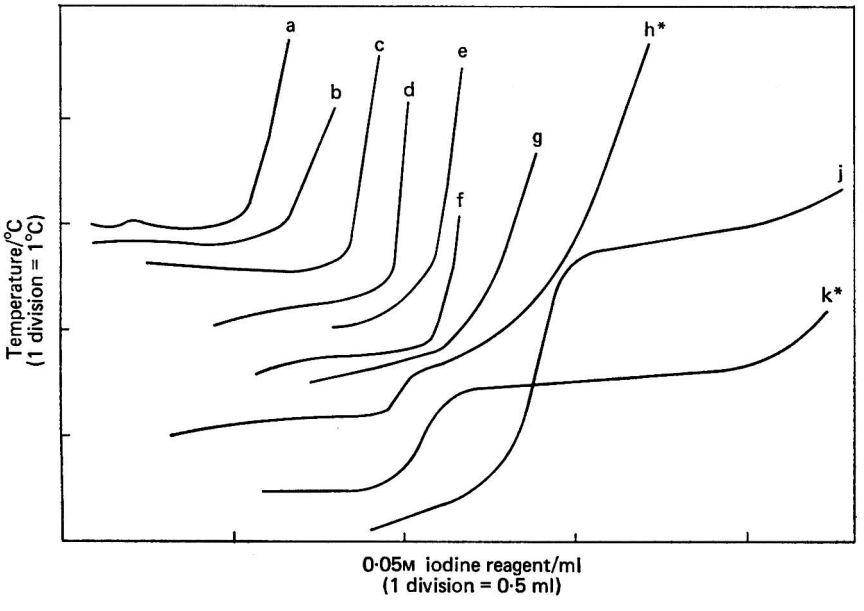
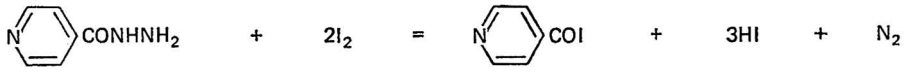


Fig. 3. Catalytic thermometric titration of hydrazine derivatives with 0·05 M iodine reagent. Compounds/mg: a, 2-hydroxyethylhydrazine, 1·1; b, *NN*-dimethylhydrazine, 0·65; c, *NN*-diphenylhydrazine, 9·2; d, 4-nitrophenylhydrazine, 5·1; e, 2,4-dinitrophenylhydrazine, 20·2; f, benzoylhydrazine, 3·4; g, isonicotinoylhydrazine, 0·85; h,* phenylhydrazine, 11·0; j, 4,4′-oxybis(benzenesulphonohydrazide), 26·6; and k,* benzenesulphonohydrazide, 28·9.

* Titrant, 1 division = 0·83 ml; temperature, 1 division = 2·0 °C

and phenylhydrazine, iodine is consumed after the first sharp inflection in the titration graph, which has been taken as marking the end-point of the titration. It is probable that a slow evolution of nitrogen occurs after the initial reaction with iodine and, ultimately, four atoms iodine would be required for each hydrazine group. Any further reaction with nitrogen evolution would, of course, be accelerated by the rising temperature caused by the ionic polymerisation.

Observations (*b*) and (*c*) above suggest that, in dimethylformamide, iodine reacts with the heterocyclic nitrogen as well as with the hydrazine group of the isonicotinoylhydrazine. If the reaction of the 4·2 atoms of iodine with each molecule of isonicotinoylhydrazine occurred only at the hydrazine group, the reaction could be explained essentially by an equation similar to that which applies to the reaction in aqueous solution:



In this instance a molecule of nitrogen would be liberated from each molecule of hydrazide.

Gasometric experiments with hydrazides have been carried out to determine whether the iodine reactant is consumed in reactions involving the evolution of a gas. Aliquots (1 ml) of a solution of isonicotinoylhydrazine in dimethylformamide were added to an excess of iodine in 5 ml of dimethylformamide at ambient temperature (25 °C in this experiment) and the volume of nitrogen evolved after the addition of each aliquot was measured. The experiment was then repeated with benzoylhydrazine. The results of the experiment are summarised in Fig. 4. With both hydrazides, the rate of evolution of nitrogen after addition of the second aliquot was almost constant until five aliquots had been added, when it began to decrease. During the "steady state," the rate of nitrogen evolution was about the same for both hydrazides, 0·6 mmol of nitrogen being released from each millimole of hydrazide, which is equivalent to a reaction ratio of 2·4 atoms of iodine to 1 molecule of hydrazide if two molecules of iodine are required for the release of one molecule of nitrogen.

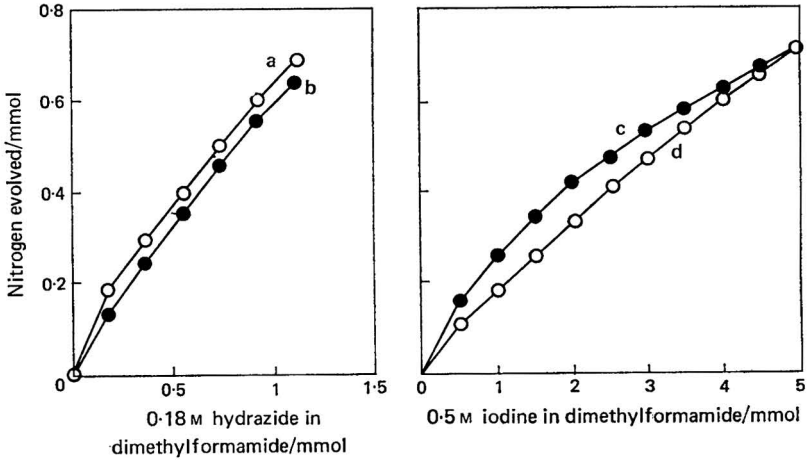


Fig. 4. Gasometric measurements in the reaction of isonicotinoylhydrazine and benzoylhydrazine with iodine in dimethylformamide solution: a, addition of 0·18-mmol aliquots of isonicotinoylhydrazine to 4 mmol of iodine; b, addition of 0·18-mmol aliquots of benzoylhydrazine to 4 mmol of iodine; c, addition of 0·5-mmol aliquots of iodine to 4 mmol of benzoylhydrazine; and d, addition of 0·5-mmol aliquots of iodine to 4 mmol of isonicotinoylhydrazine

The experimental conditions are not the same as those obtaining in the iodimetric titration because the ethyl vinyl ether is omitted for obvious reasons. However, it is reasonable to assume that the reactivities of the two hydrazides are similar when measured by the amount of nitrogen evolved and that the much greater consumption of iodine by isonicotinoylhydrazine,

4·2 atoms compared with 1·4 atoms per molecule, is caused by a reaction involving the heterocyclic nitrogen of the latter compound.

When the reverse procedure is carried out, *i.e.*, when 1-ml aliquots of iodine are added to an excess of the hydrazide in dimethylformamide, nitrogen is evolved immediately following addition of the first aliquot in both instances (Fig. 4). Thus, there is no evidence from this experiment that with isonicotinoylhydrazine a reaction with the heterocyclic nitrogen takes precedence over a reaction with the hydrazide group.

With all of the samples examined, except water, calibration graphs proved to be linear in the range 0 to 1 ml of titrant when titrant was added at rates of 0·05 to 0·2 ml min^{-1}. The effect of the rate of titrant addition on the calibration graph for isonicotinoylhydrazine is shown in Fig. 5. Increasing the rate of addition of the titrant can be seen to displace the linear calibration, but, because the lines are parallel, the calibration factor remains constant

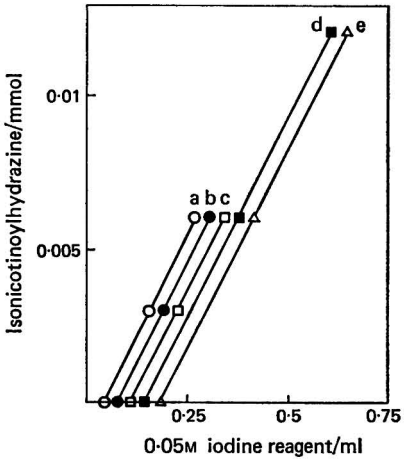


Fig. 5. Effect of the rate of addition of titrant on calibration graphs in the thermometric titration of isonicotinoylhydrazine with 0·05 M iodine reagent. Rate of addition of titrant/ml min^{-1}: a, 0·02; b, 0·032; c, 0·06; d, 0·12; and e, 0·20

The sharp inflection at the end-point with most of the titrations gives this catalytic thermometric method a slightly higher precision than the corresponding acid - base titrations described in Parts I[5] and II,[9] in which the 0·05 M titrant was used. With this titrant, coefficients of variation of less than 1 per cent. have been obtained in most of the titrations. With 0·01 M titrant, sharp end-point inflections were still obtained but the precision was of the order of 2 per cent. Some precision values are given in Table III. Titration graphs obtained with 0·01 and 0·005 M titrants are shown in Fig. 6.

A number of solvents have been examined as possible alternatives to dimethylformamide. Toluene, *NN*-dimethylacetamide, 1,2-dichloroethane, tetrahydrothiophene 1,1-dioxide (sulpholane), propylene carbonate and 2-methoxypropionitrile can be used as solvents for the iodine titrant or for the sample. Toluene gives less sharp end-points than does dimethylformamide with some compounds and the latter solvent has been preferred in the present study.

Several alkyl vinyl ethers were assessed before ethyl vinyl ether was chosen as the monomer for this detailed investigation. The alternatives, n-butyl vinyl ether, isobutyl vinyl ether, 2-chloroethyl vinyl ether and divinyl ether, all proved to be inferior to the chosen monomer. No discernible end-points were obtained with the last two ethers but n-butyl and isobutyl vinyl ethers gave acceptable end-points in the titration of amines (Fig. 7).

As interhalogen compounds, such as iodine bromide and iodine chloride, have been used as titrants in oxidation - reduction reactions, some of these compounds, namely iodine bromide, iodine chloride and iodine trichloride, have been tried as alternatives to iodine

TABLE III

RESULTS FOR PRECISION FROM THE THERMOMETRIC TITRATION OF ORGANIC BASES, HYDRAZINE DERIVATIVES AND WATER WITH 0·05 AND 0·01 M IODINE SOLUTION IN DIMETHYLFORMAMIDE*

Compound	Amount taken/mg	Titrant molarity†	*n*‡	Mean titre/ml	Standard deviation	Coefficient of variation, per cent.
Pyridine	1·03	0·05	4	0·49	0·0025	0·51
Tris(hydroxymethyl)methylamine	1·20	0·05	3	0·62	0·0054	0·88
NN-Dimethylhydrazine	0·86	0·05	4	0·44	0·0026	0·59
Benzoylhydrazine	6·64	0·05	3	0·66	0·0040	0·61
Isonicotinoylhydrazine	1·70	0·05	4	0·38	0·0024	0·63
Benzenesulphonohydrazide	28·9	0·05	3	0·55	0·0058	1·06
Thiosemicarbazide	2·34	0·05	3	0·70	0·0082	1·18
Water	17·0	0·05	4	0·66	0·0045	0·68
Tris(hydroxymethyl)methylamine	0·24	0·01	3	0·55	0·010	1·82
Isonicotinoylhydrazine	0·34	0·01	3	0·54	0·0035	0·64
α-Picoline	0·28	0·01	3	0·59	0·0064	1·07

* By thermometric procedure; titrant added at 0·12 ml min^{-1}.
† Nominal value.
‡ Number of determinations.

in solution in dimethylformamide for the thermometric titrations. All three of these interhalogen compounds gave inferior titration graphs to that of iodine in the titration of pyridine, although all gave rises in temperature of the same order as that obtained with iodine.

Although the iodimetric titration method is suitable for the assay of single compounds when interfering substances are absent, the method is obviously unsatisfactory for the determination of the total amount of a particular functional group in a complex mixture because different compounds combine with iodine in different molar ratios. However,

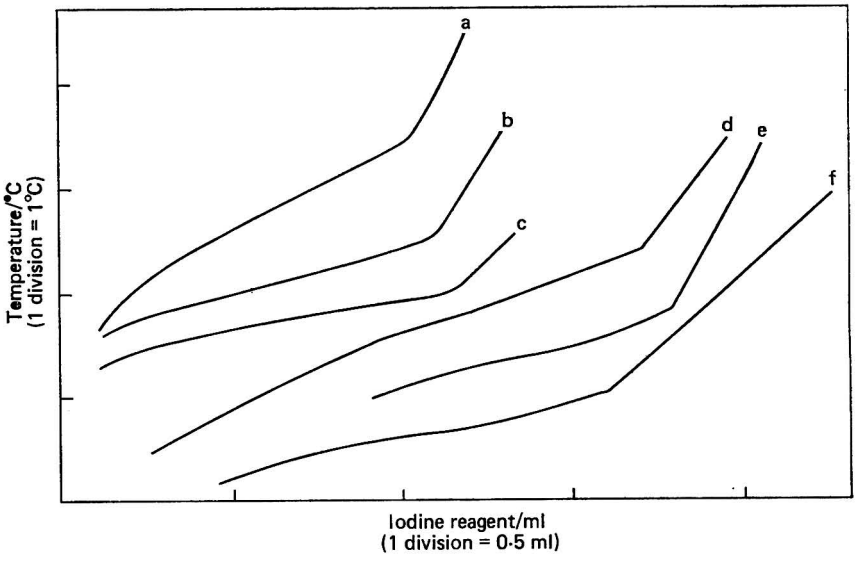


Fig. 6. Catalytic thermometric titration of organic bases, hydrazine derivatives and water with 0·01 and 0·005 M iodine reagents.

	a	b	c	d	e	f
Compound/mg	A,0·54	B,0·24	C,0·28	D,0·14	E,2·0	E,1·0
Titrant/M	0·01	0·01	0·01	0·005	0·01	0·005
Recorder/mV range	50	100	100	50	100	100

Compounds: A, isonicotinoylhydrazine; B, tris(hydroxymethyl)methylamine; C, α-picoline; D, pyridine; and E, water

differences in reaction ratios make it possible to determine selectively the components of binary mixtures. A calibration graph for the analysis of a mixture of benzoylhydrazine and isonicotinoylhydrazine is shown in Fig. 8.

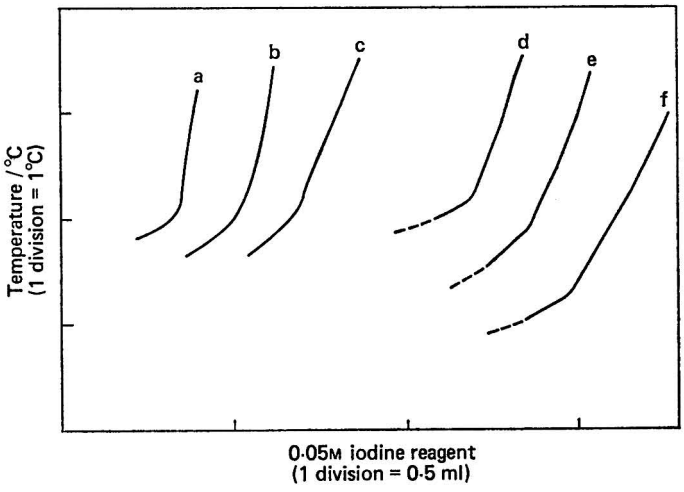


Fig. 7. Effect of monomer on end-point sharpness in blank titrations and in titrations of pyridine with 0·05 M iodine reagent. a, b and c, blank titrations (1 ml of dimethylformamide); d, e and f, titrations of pyridine (0·01 mmol). Monomers: a and d, ethyl vinyl ether; b and e, n-butyl vinyl ether; and c and f, isobutyl vinyl ether

The results of titrations carried out with organic bases suggest that the number of iodine atoms that combine with one molecule of the base can be used as a measure of the strength of the base in the solvent used. In addition, the widely differing reactivities of the various compounds examined should make it possible to use the iodimetric titration in order to elucidate the structure of more complex heterocyclic nitrogen compounds and hydrazine derivatives. With bases, such a procedure for investigation of structure could be used in conjunction with that proposed in Part I.[5]

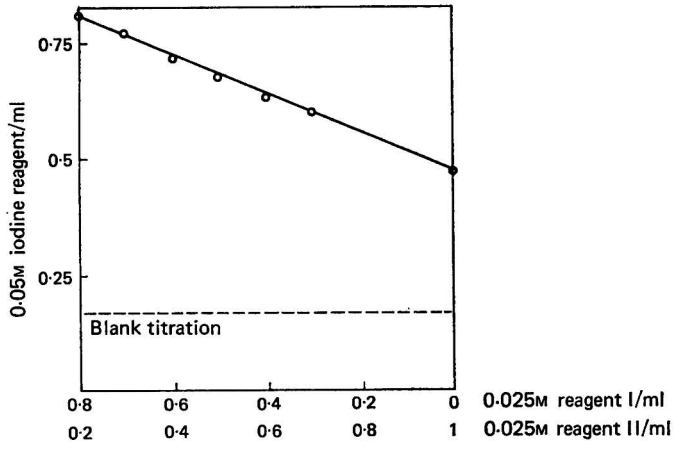


Fig. 8. Calibration graph for the determination of benzoylhydrazine and isonicotinoylhydrazine in binary mixtures by catalytic thermometric titration with 0·05 M iodine reagent. Reagent I, isonicotinoylhydrazine in dimethylformamide; reagent II, benzoylhydrazine in dimethylformamide

The fact that, for many compounds, this iodimetric method gives results which indicate that iodine combines with the titrand in fractional molar amounts should not detract from its value as an analytical method. The requirements of reproducibility of results and linearity of calibration graphs can be met.

Fisons Limited, Agrochemical Division, are thanked for gifts of chemicals and Mr. S. F. George is thanked for the construction of apparatus.

References

1. Prescott, A. B., and Trowbridge, P. F., *J. Amer. Chem. Soc.*, 1895, **17**, 859.
2. Coffey, S., *Editor*, "Rodd's Chemistry of the Carbon Compounds," Second Edition, Volume 1, Part B, Elsevier Publishing Company, London, 1965, p. 128.
3. Cheronis, N. D., and Ma, T. S., "Organic Functional Group Analysis by Micro and Semimicro Methods," Interscience Publishers, New York, 1964, p. 289.
4. Siggia, S., *Analyt. Chem.*, 1948, **20**, 762.
5. Greenhow, E. J., and Spencer, L. E., *Analyst*, 1973, **98**, 81.
6. Greenhow, E. J., *Chem. & Ind.*, 1973, 697.
7. Greenhow, E. J., and Spencer, L. E., *Analyst*, 1973, **98**, 98.
8. Dixon, J. P., "Modern Methods of Organic Microanalysis," Van Nostrand Co. Ltd., London, 1968, p. 211.
9. Greenhow, E. J., and Spencer, L. E., *Analyst*, 1973, **98**, 90.

Note—References 5, 7 and 9 are to Parts I, III and II of this series, respectively.

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Problems in the Determination of Carbon in Steel by a Precision Coulometric Method

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An apparatus for the coulometric determination of carbon in steel with a precision approaching 1 part in 1000 at the 1 per cent. level has been constructed. The measures needed to overcome sources of error in gas absorption, pH measurement, control and coulometric measurement are described. The design avoids the use of a gas proportionating pump and can be used with a 0·2-g sample containing up to 2 per cent. of carbon, and proportionately larger amounts up to 2 g for lower carbon contents. Results on a series of standard steels are given.

A RECENT paper by Boniface and Jenkins[1] has summarised the choice of methods currently available for the determination of carbon in steel and given reasons for the choice of the coulometric principle for development as a reference method. A similar need arose in this laboratory from the tendency towards narrower specification limits for carbon in low-alloy steels intended for automated heat treatment in the engineering industry.

From private information about the work of the Carbon Study Group of the Analysis Committee of B.I.S.R.A., later described by Scholes at the 23rd Chemists' Conference,[2] it became clear in 1968 that the coulometric method was capable of producing results of excellent precision and had the advantage of being an absolute method, independent of chemical standardisation. Visits to laboratories in Germany confirmed these impressions but although two types of apparatus have been described in the literature,[3–5] which are now in commercial production, they have not become popular in the United Kingdom, possibly because of their relatively high cost. However, a simple manual coulometric procedure has been given[6] as a German standard procedure for steels with less than 0·1 per cent. of carbon.[5]

A later version dealing with all classes of iron, steel and ferro-alloys is now described in a supplementary volume,[7] but this standard refers only to the use of commercially available apparatus operated according to the maker's instructions.

The rapidly decreasing cost of modern integrated circuits and digital displays makes electronic integration an attractive basis for precision measurement. It was therefore decided to develop our own automatic coulometric carbon-in-steel analyser, and this paper describes some of the little known problems encountered, and their solution.

CHOICE OF EXPERIMENTAL CONDITIONS

Difficulties with retention of carbon dioxide in the coulometric cell have caused previous workers to limit the carbon content of the specimen to a maximum of 1 mg[1,4,5] and often to as little as 0·2 or 0·3 mg.[3,6] An expensive precision proportionating pump, usually of 1:9 ratio has been needed to deal with higher carbon levels[3,4,7]; indeed Thomich[8] has advocated the use of an additional 1:4 ratio pump for carbon contents greater than 1·5 per cent.

In the manual titration methods of Boniface and Jenkins and of the Verein Deutscher Eisenhüttenleute, timing of a fixed coulometric titration current of 20 mA is used. One of the commercial instruments[3] uses currents up to 321·2 mA integrated by a low-friction current motor in earlier versions, and electronic integration later; the other[5] uses a count of variable frequency pulses of 100·5 mA from a known capacitor.

Our aim was to develop a method with a precision of 1 part in 1000 at the 1 per cent. of carbon level, *i.e.*, a sensitivity of better than 2 μg, and capable of dealing with up to 2 per cent. of carbon without the need to use a precision proportionating pump. A typical analytical balance has a tolerance of 0·1 mg, so the amount of sample could not be reduced below 0·2 g without using a micro-balance, which would have been an undesirable restriction. The proposed apparatus had therefore to be capable of handling up to 4 mg of carbon, and it was obvious that there would be a serious problem in ensuring retention of carbon dioxide

in the absorption cell. Much higher coulometric titration currents would also be required, because 4 mg of carbon would require 64 C, equivalent to an average of just over 0·5 A, if generated steadily for 2 minutes.

The absorption - coulometric titration system chosen for study was based on that described by Abresch and co-workers[3,9] in which the reagent used is alkaline barium perchlorate, rather than barium chloride, which avoids the generation of chlorine as a side reaction. End-point detection was chosen to be a combined glass - calomel rather than a platinum electrode in weak hydrogen peroxide solution[3,6] because the former allowed a much simpler glass cell assembly to be constructed, although it was known that this choice might introduce problems from electrical interference in the high-impedance circuits.

In order to develop a method capable of measuring down to 1 part in 1000, the first experiments comprised investigations into the precision that would be needed in measuring or maintaining variables of the absorption - coulometric titration cycle. On the assumption of nine randomly distributed contributory errors, each would have to be kept below about 1 part in 3000.

Following German practice,[3,6] an experimental cell was set up containing 140 ml of 5 per cent. *m*/*V* barium perchlorate solution, and 0·033 N sodium hydroxide solution was added from a 10-ml burette, the changes in pH monitored by a conventional pH meter being noted. Each 1 ml of standard alkali solution was equivalent to 200 μg of carbon or 0·1 per cent. in a 0·2-g sample. The curve obtained by plotting carbon equivalent against pH, and against millivolts output from the combined electrode, allowed the rate of change of pH and millivolts to be determined. In similar experiments, barium hydroxide was added or generated electrolytically and a graph of pH or electrode millivolts against millicoulombs was established. Fig. 1 shows the form of the curves. It was anticipated that the coulometric cell would generate heat, necessitating temperature control, and the titrations showed the curves to be of similar shape at room temperature and at 37 °C.

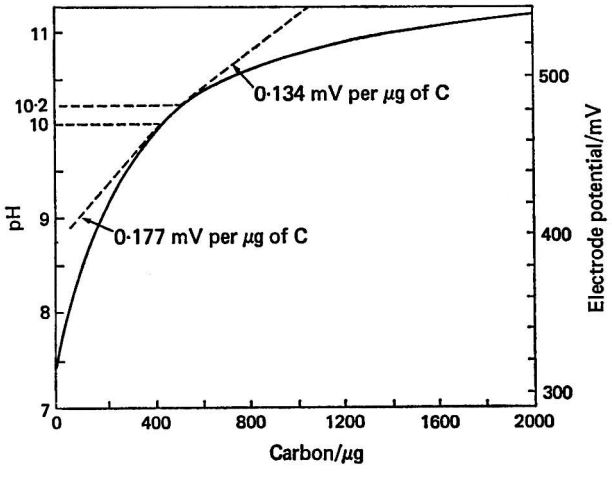

Fig. 1. Relationship between pH, electrode potential and equivalent carbon content in a 140-ml coulometer cell using an electrode with E_0 at pH 2

From the curve it is clear that the inflection and true end-point are close to pH 7, but in this region absorption of carbon dioxide would be expected to be very incomplete. The relevant literature suggested that other workers had found a pH of about 10 to 11 to be necessary for complete absorption.[3,9] Reading from the graph, for the cell containing 140 ml of 5 per cent. *m*/*V* barium perchlorate solution, at pH 10·2, the determination of carbon with a precision of 2 μg required the operating end-point error to be less than 0·0046 pH unit, equivalent to an error of less than 0·268 mV from the electrode system in the absence of any other variables.

Experiments with this cell, initially with manual titration and later with automatic titration, produced satisfactory results with 1·4 mg of carbon (0·7 per cent. on a 0·2-g sample), but with higher carbon contents reproducibility and precision of results were unsatisfactory.

During these early experiments, a number of problems were encountered that would have to be resolved in the determination of carbon in steel up to the 4-mg level without the use of fractionating devices. The necessary solutions were found concurrently during the development period, but for clarity each problem, and the modification leading to the final design of the apparatus, is described separately.

Factors to be considered

Chemistry and electrochemical equivalence
Temperature control
Absorption of carbon dioxide
pH electrode
Coulometer cell
Coulometer
Combustion conditions
Other factors affecting the precision of the method

Chemistry and electrochemical equivalence—

The steel is burnt in an excess of oxygen and its carbon content converted into carbon dioxide, which is absorbed in the cathode compartment of the coulometric cell, thus precipitating barium carbonate and liberating the equivalent amount of hydrogen ions. The original pH is restored by electrolysis. Many reactions are possible but the following indicate the electrical equivalents and formation of the final products.

Cathode compartment—

Absorption:

$$Ba^{2+} + CO_2 + H_2O \rightarrow \underset{\text{(precipitate)}}{BaCO_3} + 2H^+$$

Electrolytic titration:

$$2H^+ + 2e^- \rightarrow \underset{\text{(gas)}}{H_2}$$

Anode compartment—

Electrolysis:

$$H_2O \rightarrow 2H^+ + \tfrac{1}{2}\underset{\text{(gas)}}{(O_2)} + 2e^-$$

Chemical neutralisation:

$$2H^+ + BaCO_3 \rightarrow Ba^{2+} + \underset{\text{(gas)}}{CO_2} + H_2O$$

Thus in this process, one carbon atom $\equiv 2e^-$

$$12{\cdot}010\text{ g of carbon} \equiv 2F \equiv 2 \times 96\,495\text{ C}$$

by using the value for the Faraday obtained by Craig and Hoffman,[10] from which 4 mg of carbon correspond to 2·0 per cent. in a 0·2-g sample and 64·276 C.

Temperature control—

The change in potential from a pH-sensing electrode for a change of 1 pH unit is given by

$$E = \frac{2{\cdot}303\,RT}{F}\text{ V}$$

From this equation, the electrode e.m.f. depends upon the absolute temperature; substitution of the appropriate constants gives a rate of change of e.m.f. of 58·16 mV per pH unit at 20 °C, but 62·13 mV per pH at 40 °C.

For older electrodes, the output is usually zero at about pH 2; for modern electrodes the potential is usually arranged to be zero at about pH 7.

The effect of temperature on the apparent pH is illustrated in Fig. 2. With a combined electrode that has E_0 at pH 2, to maintain the potential within 0·268 mV (equivalent to 2 μg of carbon in a 140-ml cell) at pH 10·2 requires the temperature stability to be better than 0·165 °C; with an electrode with E_0 at pH 7 the temperature problem is eased to maintaining temperature stability within 0·422 °C. However, each of the contributory errors must be one third of these values, equivalent to 2/3 μg of carbon, and as the cell volume was subsequently increased to 350 ml, the required temperature stability with an electrode with E_0 at pH 7 had to be better than 0·055 °C (see Absorption of carbon dioxide).

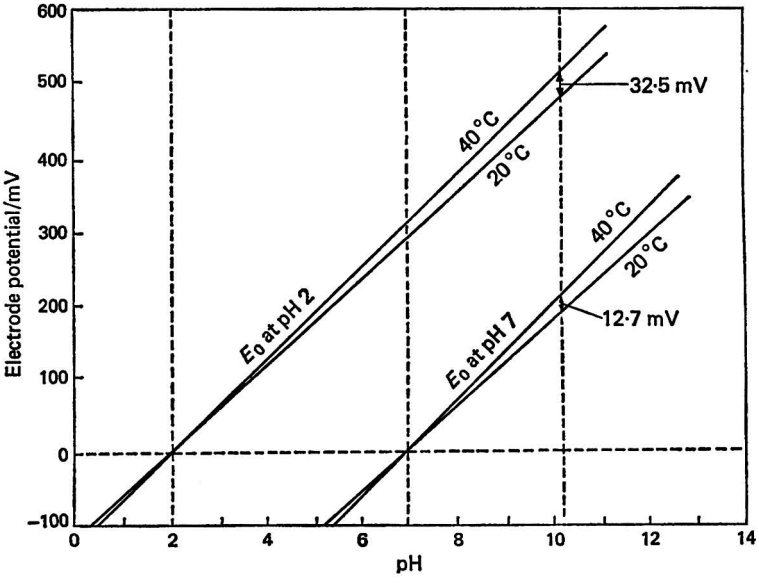

Fig. 2. Effect of E_0, pH and temperature on the electrode e.m.f.

Some rough measurements showed that the resistance of the cell might be expected to be about 100 Ω. Assuming the worst case of 2 per cent. of carbon from a 0·2-g sample of steel, and the electrolysis spread over about 2 minutes at an *average* current of just over 0·5 A, the heat input to the cell would be 825 cal, causing a rise of about 5·9 °C in 140 ml of electrolyte or 2·4 °C in 350 ml. In order to avoid the added complication of a water-cooling system, we decided to run the cell above room temperature.

The heat dissipated in the cell during the high-current phase of a titration is removed by a vertical current of air at room temperature. The air enters the case of the magnetic stirrer motor from a small blower, and leaves via a ring of holes surrounding the base of the glass coulometer cell.

During standby conditions and at the end of a titration the cell is maintained above room temperature by a small electrical resistance heater immersed in the electrolyte, and controlled by a sensing device also in the cathode compartment.

A practical difficulty was to produce a heater of sufficient power output, yet of sufficiently low thermal capacity and small physical size to give rapid control, and efficiently insulated to withstand the rather aggressive alkaline barium perchlorate solution.

The original design was a 25-W wire-wound heater immersed in silicone oil in a glass envelope. This heater had a large time constant and was unable to maintain the cell temperature within the specified limits when absorbing the carbon dioxide from 4 mg of carbon.

A new design with a printed circuit heater enclosed in a shrinkable silicone sheath had a smaller thermal equivalent, better conductivity and a much smaller time constant. It also gave a higher power output with little change in physical size. The improvement in control with the new heater is shown in Fig. 3, which also illustrates the advantage in operating

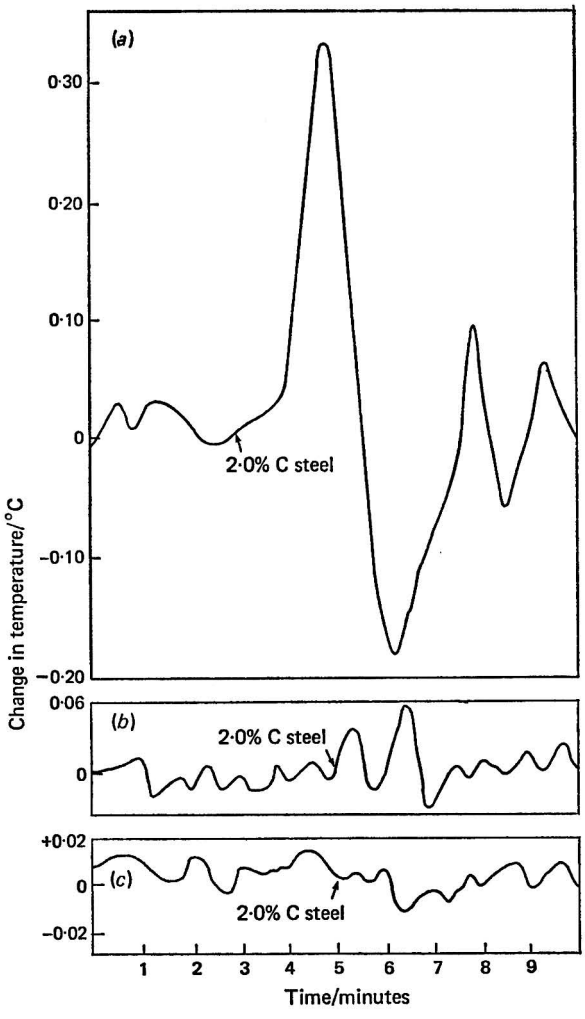

Fig. 3. Effect of heater design on temperature control of coulometer: (*a*), original heater (room temperature 20·5 °C, cell at 32·9 °C; (*b*), redesigned heater (cell at 32·9 °C); and (*c*), redesigned heater (cell at 40 °C)

the cell with a greater cooling differential. From a study of cooling curves of the cell electrolyte it was concluded that a temperature differential of at least 5 °C between the cell operating temperature and ambient room temperature was desirable. Eventually, it was decided that the optimum operating temperature would lie in the range 35 to 37 °C.

The temperature control system that has been developed monitors by means of a thermistor in the input circuit of a transistorised amplifier. The maximum output of about 2·5 A into the heater of low thermal capacity enables up to 42 W to be applied to the cell. The time constant is very small and the temperature is maintained steady within $\pm$0·015 °C, which is within the range required by other parameters.

ABSORPTION OF CARBON DIOXIDE—

Gas dispersion—A major problem with cells for the absorption of carbon dioxide is to produce a very fine gas dispersion, and in the past this has been achieved by the use of high-speed reciprocating or spinning gas distributors,[3] or by other complicated mechanical means. The need is for either very small bubbles or for a long path length in the electrolyte,

or a convenient compromise. Our cell is designed to use a simple encapsulated stirrer, driven magnetically from below the cell, together with gas distribution through a porous glass frit.

The problem of producing fine gas bubbles was investigated photographically by using electronic flash. It was found that grade 3 glass sinters (pore size 20 to 30 μm) produced no improvement over grade 2 (40 to 60 μm) because the greater resistance to flow raised the gas pressure and the bubbles expanded to the same size on liberation. The addition of an agent for causing frothing and reducing surface tension was found to be helpful. Methanol, ethanol, butanol and pentanol were tried, and also commercial wetting agents, but the traditional addition of propan-2-ol[3,9] was shown to be the most effective in reducing mean bubble size and suppressing the occasional formation of large bubbles. Additions from 0·1 per cent. to the normal 1 per cent. were tried; no advantage was found with concentrations greater than 0·3 per cent.

Fig. 4 shows portions of typical high-speed photographs, from which it can be seen that most of the bubbles have a diameter of about 0·01 inch with the selected conditions. Efficient absorption of carbon dioxide is improved as the period in contact with the absorption solution is increased, therefore a long bubble path is desirable.

A stroboscope was used to investigate the action of the magnetic stirrer. Although the manufacturer gave a maximum speed of 1250 r.p.m., it was discovered that eddy currents induced in its steel cover-plate were in fact reducing the true speed to 750 r.p.m. When a non-magnetic top-plate was fitted to the stirrer, speeds up to 2100 r.p.m. could be obtained. An immediate improvement in carbon dioxide absorption was found, and the stirrer is now run at 1800 r.p.m. Improvements in stirring speed and cell arrangement also allowed the use of a grade 1 glass sinter gas distributor, with a corresponding drop in back-pressure in the pumping system, together with a reduced tendency to blockage of the distributor by precipitated barium carbonate.

Cell electrolyte—In the original cell containing 140 ml of 5 per cent. *m*/*V* barium perchlorate solution, with a maximum current of 0·7 A, not more than 2 mg of carbon could be retained completely during a rapid combustion, and an improved circuit capable of delivering 1·8 A was still inadequate for titrating 4 mg of carbon because of an excessive reduction in pH before recovery during coulometric titration.

The possibility of slowing down the introduction of carbon dioxide was rejected because of the resulting long titration times; after much experimentation the volume of the cell was increased to 350 ml. During this work, various combinations of barium perchlorate electrolyte at 5, 10, 20, 30 and 40 per cent. *m*/*V* concentration were tried as anolyte and catholyte. The slope of the titration curve was greatly reduced at high concentration, which correspondingly increased the problems of temperature control and pH measurement. Low concentrations in the anode compartment appeared to be unable to supply the barium-ion migration required by high currents, and the use of different concentrations for anolyte and catholyte caused problems due to diffusion.

Eventually the use of 20 per cent. *m*/*V* barium perchlorate solution was adopted as the best compromise, allowing high currents, and giving complete absorption of carbon dioxide with adequate sensitivity at the end-point. The effect of barium perchlorate concentration on the sensitivity of the pH - coulomb equivalent curve can be seen in the two typical graphs of Fig. 5, in which small changes in choice of end-point cause large differences in the sensitivity of detection required.

Raising the cell operating temperature, with other conditions remaining constant, also decreases the slope of the pH - coulomb equivalent curve, two typical examples being shown in Fig. 6. Here the millivolt output from the sensing electrode alters both its absolute value and its temperature - pH coefficient and these variables also affect the sensitivity required for end-point detection. A few of the results accumulated in the experiments to assess the minimum pH required for complete carbon dioxide absorption are given in Table I, and are for a cell containing 20 per cent. barium perchlorate electrolyte operating at 37 °C and with propan-2-ol additions to aid the formation of fine bubbles.

There was no significant loss of reproducibility until the end-point had been lowered below pH 9·35. The improved circuits and solenoid valve described in other sections now prevent the sensing electrode potential from falling more than 10 mV, equivalent to 0·16 pH unit, even with very rapid absorption of carbon dioxide equivalent to 4 mg of carbon, thus allowing the choice of pH 9·5 as the operating point with the cell conditions described above.

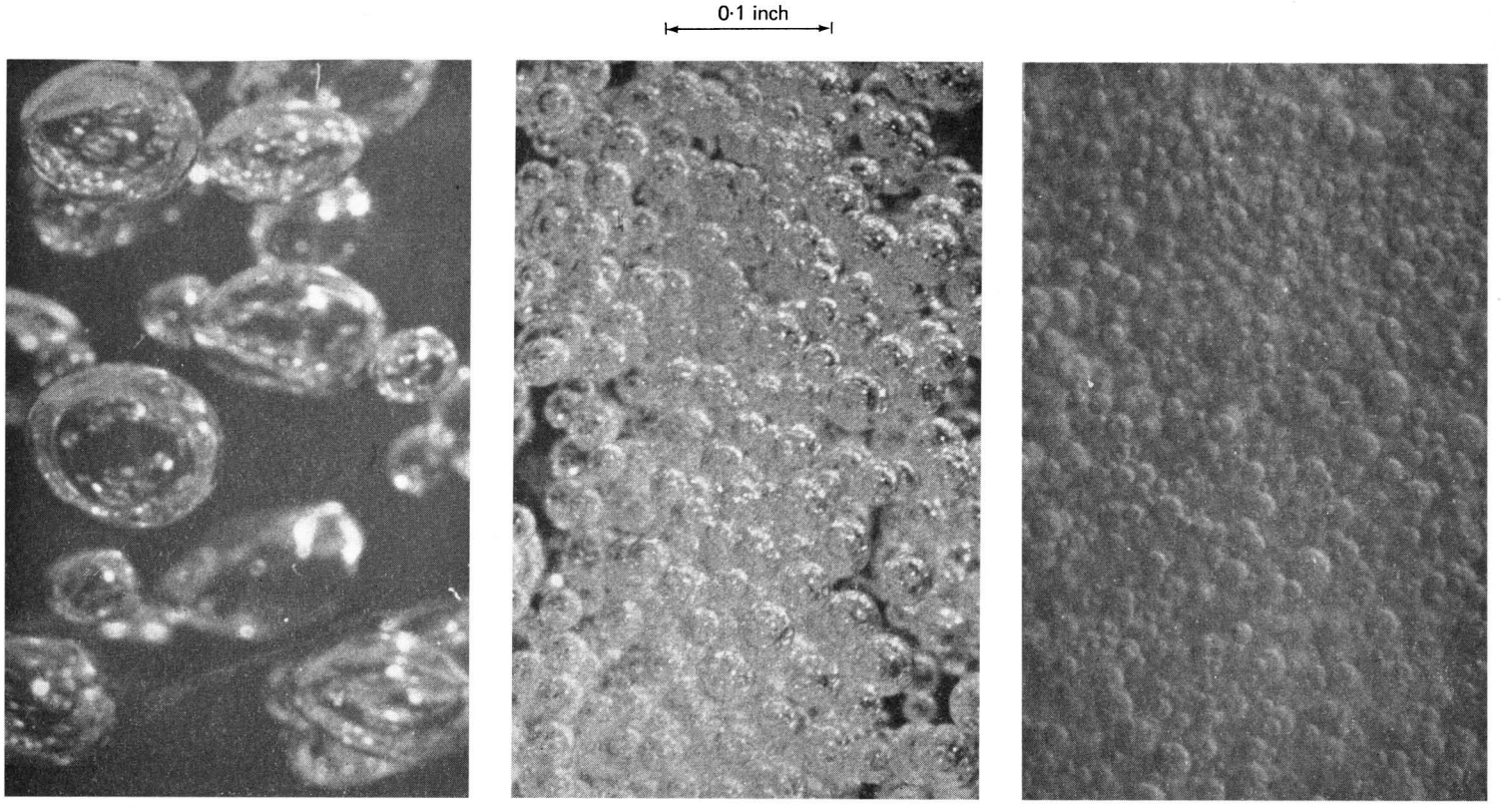

Fig. 4. High-speed photographs of bubble distribution from grade 1 glass frit. Effect of addition of propan-2-ol and stirring: (*a*), water only; (*b*), with 0·3 per cent. of propan-2-ol; and (*c*), with 0·3 per cent. of propan-2-ol, stirred

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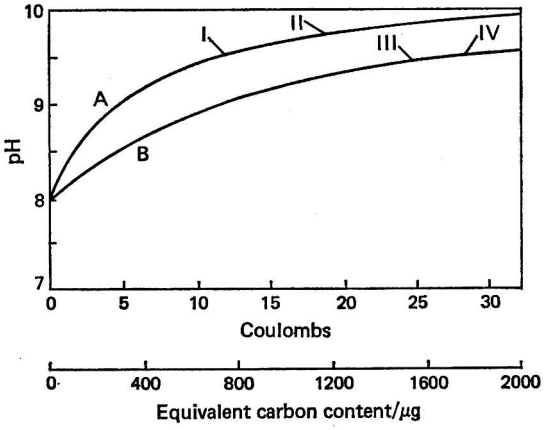


Fig. 5. Effect of barium perchlorate concentration on the relationship between pH and coulometric carbon sensitivity at 37 °C. Barium perchlorate concentration: A, 10 per cent. m/V; and B, 30 per cent. m/V. Points I to IV—

Point	pH	mV	mV ≡ 1 μg of C
I	9·52	195	0·037
II	9·69	205	0·024
III	9·44	190	0·022
IV	9·52	195	0·018

pH ELECTRODE—

It is obvious that the precision of the instrument is not only dependent on the ability to detect small changes in the potential of the electrolyte, but also on the stability of the mV - pH output of the pH electrode. Therefore, much investigation into the choice of the most suitable electrode has been needed.

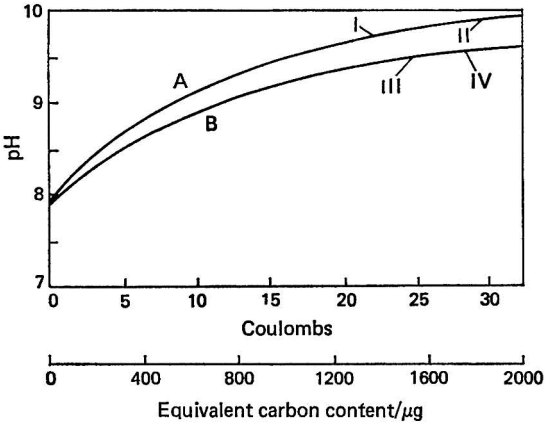


Fig. 6. Effect of operating temperature on the relationship between pH and coulometric carbon sensitivity. Barium perchlorate concentration, 30 per cent. m/V. Temperature: A, 22 °C; and B, 37 °C. Points I to IV—

Point	pH	mV	mV ≡ 1 μg of C
I	9·70	195	0·025
II	9·86	205	0·020
III	9·44	190	0·022
IV	9·52	195	0·018

TABLE I

EFFECT OF pH ON THE REPRODUCIBILITY OF CARBON DIOXIDE ABSORPTION

End-point		Carbon determinations		
mV	pH	No.	Mean, per cent.	Standard deviation (σ)
205	9·67	10	$1{\cdot}823_3$	$0{\cdot}0021_6$
195	9·51	10	$1{\cdot}820_7$	$0{\cdot}0025_8$
185	9·35	10	$1{\cdot}821_9$	$0{\cdot}0022_3$
175	9·19	10	$1{\cdot}811_8$	$0{\cdot}0058_3$
165	9·02	10	$1{\cdot}803_4$	$0{\cdot}0083_4$
157	8·89	10	$1{\cdot}785_9$	$0{\cdot}0048_6$

A precision equivalent to 2/3 μg of carbon at the end-point in 350 ml of electrolyte required the electrode and detection circuit to respond to 0·02 mV. Ideally, an electrode with immediate response to this order of change, and an electrolytic current generator capable of maintaining the end-point within this limit, are desirable. In practice, it is impossible to maintain the end-point pH so exactly because of the rapid combustion and evolution of carbon dioxide, a finite mixing time in the cell and the limits of current available. Therefore, some deviation in pH is inevitable, and as it is impossible to construct an electrode with an immediate response, a time constant is always present in the titration.

Early development work was carried out with a combined electrode with a robust cylindrical membrane, and porcelain-frit liquid junction to the salt bridge, which had zero output at pH 2·0. These features tended to give a rather slow response, and it was found that when high-carbon steels were analysed, with a consequent excursion of 20 to 30 mV from the end-point, the electrode system required several minutes to recover equilibrium. Study of the characteristics of the electrode, by imposing a sudden large change of pH [Fig. 7 (*a*)], showed that the high-resistance cylindrical glass membrane was responsible for the initial 3 minutes of the time constant, but thereafter the salt bridge was responsible. The glass half of the combined electrode has an overshoot characteristic, which in the cell results in the recording by the electrode of a higher pH than that of the electrolyte and the electrolysis current is proportionately reduced, producing a very long tail to the titration cycle. This delay in completing the titration is progressively aggravated as the excursion from the end-point is increased [Fig. 8 (*a*)].

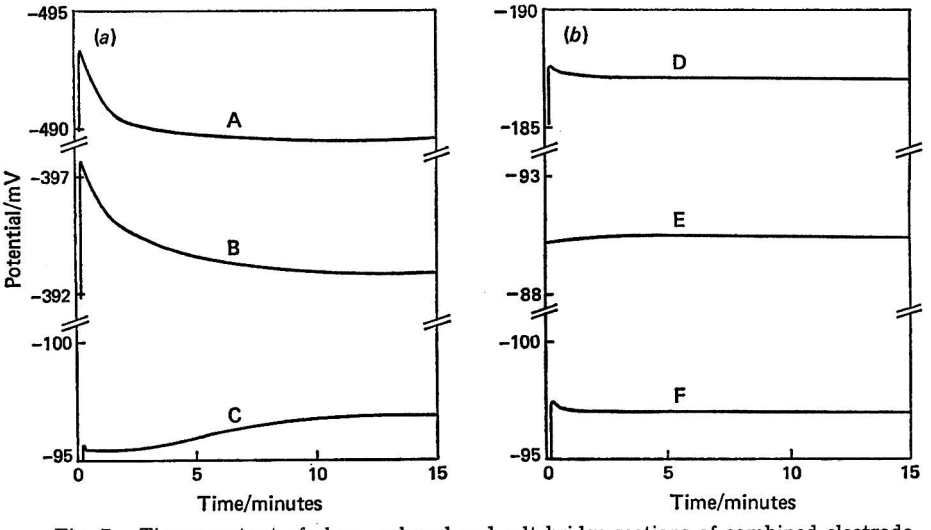


Fig. 7. Time constant of glass - calomel and salt bridge sections of combined electrode with reference to separate calomel electrode, when transferred from buffer solution of pH 7·0 to buffer solution of pH 9·0. (*a*), Robust cylindrical glass membrane, porcelain frit liquid junction: A, glass - calomel, E_0 at pH 2; B, differential; and C, salt bridge. (*b*), Low-resistance spherical membrane, taper-sleeve liquid junction: D, glass - calomel, E_0 at pH 7; E, differential; and F, salt bridge

It was obvious from these investigations that an electrode with a sensitive glass membrane and salt-bridge junction with a faster response was desirable. From the many types available, an electrode with a low-resistance glass spherical membrane and a sleeve-type liquid junction to the salt bridge, with zero output at pH 7, appeared the optimum choice.

In order to reduce the electrical interference from the coulometer and neighbouring apparatus, it was found necessary to connect the pH electrode by double-screened co-axial cable. Tests with an electrode specially made to this specification showed a remarkable improvement in response time, Figs. 7 (*b*) and 8 (*b*), and the change in E_0 reduced the effect of temperature fluctuation.

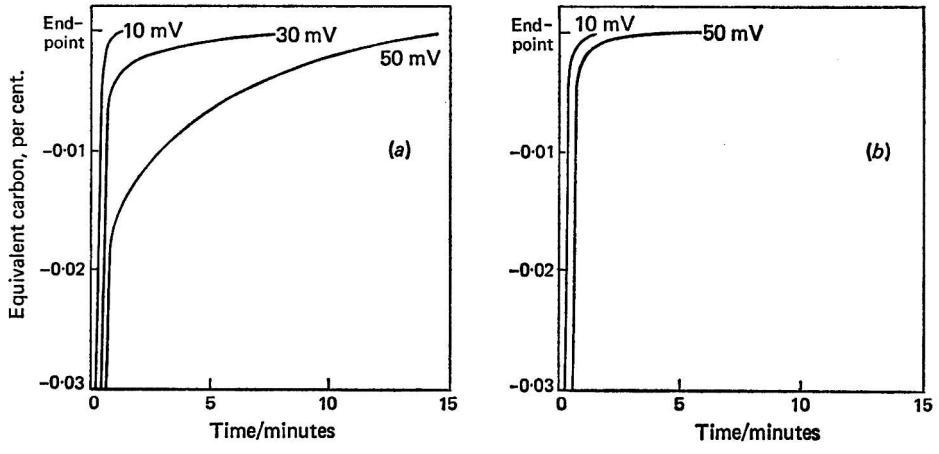


Fig. 8. Approach of titration to end-point after excursions of increasing amplitude: (*a*), E_0 at pH 2 electrode; and (*b*), E_0 at pH 7 electrode

One of the drawbacks of using a lower-resistance glass membrane is its apparent memory effect when subjected to larger changes of 2 to 3 pH units. This effect becomes apparent when the cell solution is replenished from stock solution at approximately pH 7, or if the apparatus has long periods of disuse and the pH of the electrolyte falls to 7·0. When the pH is increased to the operating pH end-point the electrode requires 1 to 2 hours to become stabilised. During the recovery period the apparatus shows a spuriously high background count. For example, after 24 hours of continuous use the background is equivalent to less than 0·0002 per cent. of carbon per minute, whereas with a newly filled cell, which has had the pH raised rapidly from 7 to 9·5, a background equivalent to between 0·0010 and 0·0020 per cent. of carbon per minute is typical. Thus it is desirable that the electrolyte should be maintained continuously at the desired end-point, even when carbon determinations are not being made.

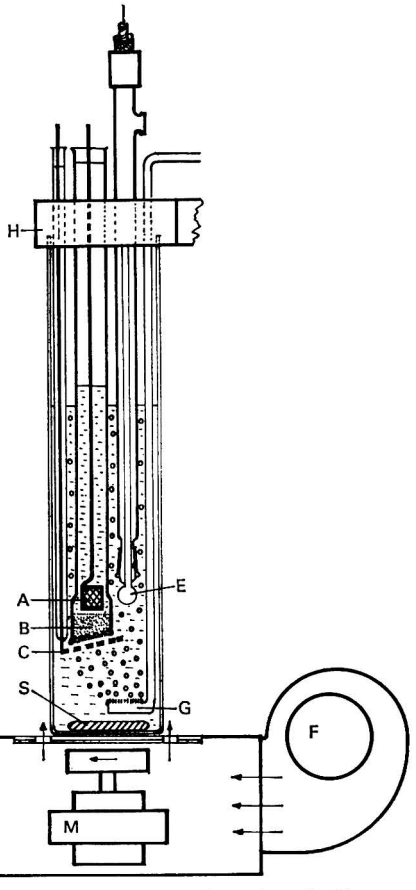
Long-term use of this type of electrode at this pH does not appear to have any detrimental effect other than a very slow change in E_0, but provided that the end-point is checked with a buffer periodically this change is unimportant. Instead of the conventional potassium chloride, 3 M sodium chloride solution is used in the electrode salt bridge in order to prevent the formation of comparatively insoluble potassium perchlorate at the liquid junction. The possibility of eliminating the salt bridge and calomel half-cell by use of a silver - silver chloride reference electrode is now being investigated.

THE COULOMETRIC CELL—

Details of the coulometric cell are shown in Fig. 9. Many such cells have been described, but the anode and cathode compartments of the electrolysis cell, and the reference and glass halves of the pH electrode, are usually separate entities joined by salt bridges and one or more diffusion diaphragms.[4–6,9] This arrangement necessitates an expensive and fragile construction and results in high resistance of the electrolysis cell with subsequent low electrolysis currents or exaggerated heating effects. These problems are avoided in our design by the

use of a concentric structure, which is largely self supporting, and by the extensive use of the O-ring seals commonly used in vacuum techniques.

The diaphragm that separates the anode and cathode compartments of the electrolysis cell was the subject of considerable experimentation. In addition to glass frits, experimental generators with anionic or cationic resins, and double glass frits with an intermediate salt bridge were tried. However, all the variants except a single glass frit had to be rejected on account of high resistance. A grade 3 frit (pore size 20 to 30 μm) of 25 mm diameter was eventually chosen as the best compromise between the needs of conductivity and retention of barium carbonate in the anode compartment.



A = Anode
B = $BaCO_3$
C = Cathode
E = pH electrode
F = Fan
G = Gas distributor
H = Head with O-rings
M = Motor
S = Stirrer

Fig. 9. Diagram of the 350-ml capacity coulometric cell (not shown—heater and temperature sensor)

The platinum electrolysis electrodes are positioned close to the diaphragm so as to keep the resistance as low as possible, but a layer of barium carbonate must be maintained between the anode and the diaphragm in order to prevent back-diffusion of acid ions into the cathode compartment.

The gas dispenser is placed close to the bottom of the cell in order to achieve a long immersion time of the carbon dioxide bubbles in the electrolyte.

Rapid recognition of any change in pH of the electrolyte is vital, as slow sensing of carbon dioxide absorption would allow a serious fall in pH before the electrolysis current could take effect, and slow reaction to the generation of hydroxyl ions would allow the cell to overshoot towards excessive alkalinity before current cut-off. For these reasons the positioning of the pH electrode is fairly critical and has been determined by experiment.

THE COULOMETER—

pH sensing unit, comparator, and electrolysis drive unit—In the initial design the pH of the cell was sensed by a combined glass - calomel electrode. Its output was amplified by an operational amplifier of very high input impedance, and was compared in a second amplifier with a standard voltage pre-set to a millivolt potential corresponding with the desired end-point pH. The signal from the comparator was fed via a transformer to the power circuit, which supplied current to the coulometer cell. For some time, satisfactory operation was achieved with this design, but eventually a breakdown in insulation occurred between the input and output circuits, causing first interference and then serious damage to the pH electrode. Because of the high impedance involved, this circuit was subject also to a.c. pick-up.

The current design illustrated in Fig. 10 involves the use of the combined glass - calomel pH electrode described above, the outputs of the two halves being fed to a matched pair of operational amplifiers, with the generator cathode electrode as the common mode. This arrangement completely isolates the electrode from the output circuit and reduces the effect of a.c. pick-up. The differential output from the amplifiers is compared in the following stage with a high-stability standard voltage pre-set to a millivolt potential corrresponding to the desired end-point pH.

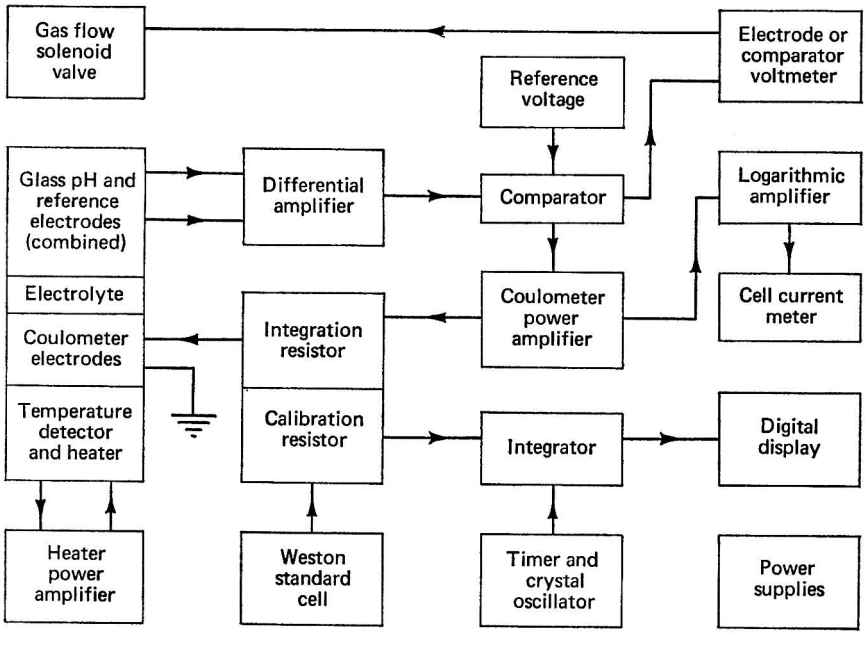


Fig. 10. Block diagram of the coulometer circuits

The third stage synchronously rectifies and amplifies the signal from the comparator and drives a power transistor supplying current to the coulometer cell. The current can reach 1·8 A when the pH of the cell deviates from the desired value.

Integrator—The potential difference developed by the electrolytic current across a high-stability resistor in the cell circuit drives an integrator circuit. In the earlier model, an

analogue integrator was used based on an operational amplifier, the output of which gave a direct reading of the percentage of carbon on the 2-V range of a 0 to 20·00-digital voltmeter. For experimental purposes, determination of the blank, or for higher sensitivity at low carbon contents, the voltmeter could be switched to a more sensitive range and measurements made to the nearest 0·0001 per cent. of carbon.

Coulometric standardisation was facilitated by measurement, with the same voltmeter, of the potential developed across a precision resistor carrying a standardising current. Precision of standardisation and results was dependent on the sensitivity and accuracy of the digital voltmeter; in the later stages of development, the 0 to 1999 output proved to have too little resolution. Another fault in this design was a stress effect in the integrating capacitor, which caused a "memory" error when low results followed high results.

The present design avoids both of these problems by using integration by means of a capacitor in which the current produces a positive charge, which is removed by a series of negative pulses of known amplitude and duration. The pulses are counted and the total is shown on a five-digit display electronically calibrated to read directly the percentage of carbon, based on a 0·2-g sample.

Standardisation—The coulometric standardisation is checked by integration of the standardising current for an accurately measured time of 200 s derived from a crystal-controlled oscillator; the standardising current is continuously checked by comparing the voltage it develops across a calibrated resistor with the potential of a permanently incorporated Weston cadmium cell at a known temperature. The calibration resistor has a temperature coefficient of 0·001 per cent. per °C and an accuracy guaranteed to be better than 0·01 per cent. Consideration of the possible variables, for example, ambient temperature and heat dissipation, gives an estimated stability of better than 1 part in 5000, and the accuracy of calibration is dependent solely on the long-term stabilities of the resistor and Weston cell, together expected to be much better than 0·1 per cent.

The calibration count is recorded on the digital display, and should have the following values calculated from the known e.m.f. and measured temperature of the Weston cell within the electronic unit:

Weston cell				Equivalent carbon	
Temperature/°C	V	Standard current/mA	C	mg	per cent.
15	1·018 79	101·879	20·3758	1·268 01	0·6340
20	1·018 61	101·861	20·3722	1·267 99	0·6339
25	1·018 39	101·839	20·3678	1·267 51	0·6338
30	1·018 12	101·812	20·3624	1·267 18	0·6336
35	1·017 81	101·781	20·3562	1·266 80	0·6334
40	1·017 46	101·746	20·3492	1·266 36	0·6332

In practice, the result has been stable within 1 part in 3000 over a period of several weeks. Any fault or drift in the integrator would become known from the display of an incorrect value when checking standardisation.

Auxiliary circuits—An auxiliary circuit from the comparator is used to operate a solenoid valve situated in the combustion gas line from the furnace, so as to cut off the flow of combustion gases to the cell if the pH of the electrolyte deviates by more than 3 mV from the end-point. This precaution is required in order to prevent large potential changes in the pH electrode; much larger excursions can be tolerated without loss of efficiency of absorption of carbon dioxide. The gas flow is reconnected when the pH of the electrolyte has recovered to the pre-set level.

A second auxiliary circuit from the comparator is used to indicate on a meter the amount of deviation from the end-point during titration. Facilities are provided in the comparator to re-set the end-point by using a buffer solution should the mV - pH relationship of the pH electrode change, or when a new electrode is fitted. A second meter displays the instantaneous coulometric current on a logarithmic scale that covers the range 100 μA to 1·0 A.

Combustion conditions—

The furnace is a conventional unit heated by three resistor rods and operated at 1320 °C. The temperature is controlled automatically by a thyristor device in the circuit of one rod.

The combustion tube is chosen to be of 15·5 mm i.d. so as to take specially made low-blank, small refractory boats with the minimum of dead-space.

Oxygen at 5 p.s.i. from a cylinder is passed through a pre-combustion tube and a soda-lime tower before being supplied to the combustion tube, near the open end, at the rate of 1·5 l min^{-1}. We have encountered trace amounts of hydrocarbons in a cylinder of oxygen on one occasion, and pre-combustion is incorporated as a precaution against possible errors from this source. Some of the oxygen and all of the carbon dioxide are withdrawn at the closed end of the furnace by a small diaphragm pump, and are delivered at the rate of 350 ml min^{-1} to the coulometer cell via the solenoid valve mentioned previously, after the oxides of sulphur have been removed with manganese dioxide. When this gas circuit is closed, oxygen, at the rate of 100 ml min^{-1}, reaches the cell via a by-pass line.

A third tube in the furnace is used to pre-burn boats at 1320 °C in an unpurified oxygen flow of 500 ml min^{-1}. To ensure low and reproducible blanks, it is essential that boats be cooled in oxygen and removed only to receive the steel sample immediately prior to the carbon determination.

The train, shown diagrammatically in Fig. 11, is constructed as far as possible from $\frac{1}{8}$ inch bore metal pipe in order to minimise dead-space. Great care is needed in the elimination of small leaks. Ingress into the suction side of the system of as little as 4 ml of air containing 0·03 per cent. of carbon dioxide would be equivalent to 0·6 μg of carbon, which is the tolerable error of the coulometer. For this reason much reconstruction of the seals on such items as the pump, flow gauges and needle valves was required.

As the method is equally sensitive to the gaseous products of sulphur combustion it is important that the removal of sulphur gases be extremely efficient.

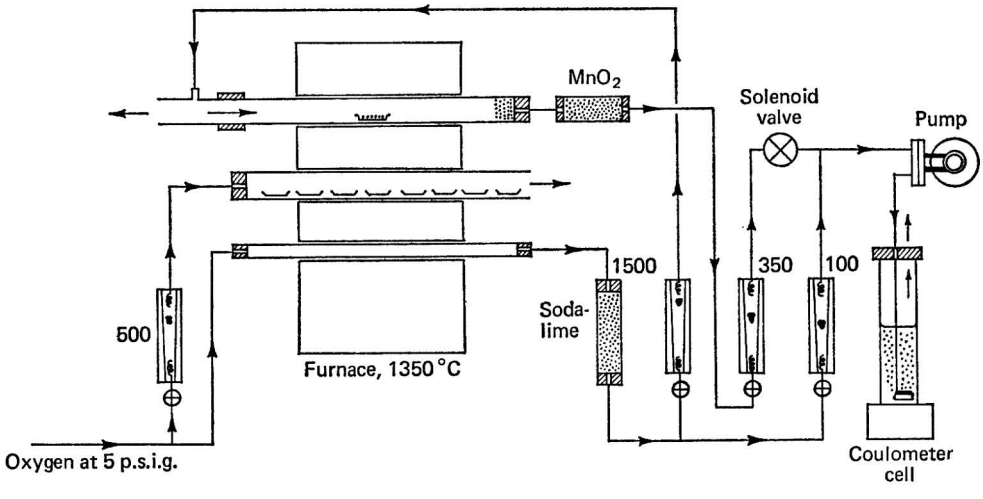


Fig. 11. Diagram of furnace train and oxygen flow system (flow-rates, ml min^{-1})

OTHER FACTORS AFFECTING THE PRECISION OF THE METHOD—

Change in pH of the cell electrolyte by loss of water—In a cell containing 350 ml of electrolyte at 37 °C, assuming a dry gas flow of 400 ml min^{-1} for a period of 6 minutes, the loss of water in saturating the gas would be 0·12 ml. This loss would produce a maximum change in pH of 0·0002 unit, equivalent to 0·0001 per cent. of carbon on a 0·2-g sample.

From the chemistry of the cell, the titration of the carbon dioxide by electrolysis results in a further loss of water: $2 \times 96\,495$ C $\equiv$ 12·01 g of carbon $\equiv$ 18 g of water; and 4 mg of carbon (2·00 per cent. on a 0·2-g sample) $\equiv$ 6 mg of water per determination, *i.e.*, 0·006 ml, which is one twentieth of the loss by evaporation and therefore negligible.

Back-diffusion in the electrolysis cell—The pH of the solution in the anode compartment depends on the solubility of barium carbonate in an acidic solution buffered by barium perchlorate and cannot be higher than about 7·0. Diffusion of this solution into the cathode

compartment would result in a reduction of the alkalinity. It is therefore convenient, although not essential, to pass a few microamperes continuously through the coulometric cell; this action serves to keep the cell polarised, produces a smoother response of the coulometer integration system near the end-point and slightly decreases the background blank.

Life of cell electrolyte—The condition of the electrolyte in the cathode compartment does not change during many weeks, apart from very small losses by evaporation, but the effectiveness of the propan-2-ol is limited. Increasing the amount of propan-2-ol has no advantage and addition of 1 ml in 350 ml of electrolyte at intervals of 2 to 3 days appears satisfactory. However, it is usual to change the electrolyte when the amount of precipitated barium carbonate becomes excessive, perhaps once per week, but this depends upon the type and number of steels analysed, and satisfactory results have been obtained over a period of a month with regular additions of propan-2-ol. In the anode compartment it is necessary only to maintain a $\frac{1}{4}$-inch thick layer of barium carbonate in order to prevent back-diffusion of acid ions.

Blank—Investigation into the use of fluxes to aid combustion was carried out. While low-alloy steels gave comparable results with or without flux, some alloy steels required the use of flux in order to ensure complete recovery of carbon. It was decided to standardise combustion by using 0·3 g of tin, which contributed the equivalent of 0·003 per cent. of carbon to the blank. If the apparatus is left continuously self-balancing on standby, the residual chemical and electrochemical blank can be kept below the equivalent of 0·4 μg of carbon per minute, *i.e.*, about 0·001 per cent. on 0·2 g of steel titrated in 6 minutes. The total reagent and electrochemical blank for a 6-minute determination, with proper care in preparation and handling of boats, selection of flux, etc., is highly reproducible, and is usually equivalent to 0·004 to 0·007 per cent. of carbon; long-term studies have given a between-day standard deviation equivalent to $0{\cdot}001_5$ per cent. of carbon.

Results obtained, future developments and comments

The B.I.S.R.A. Carbon Study Group mentioned in the introduction tested methods that are in use in twenty laboratories and depend on the following seven different principles: non-aqueous titration; thermal conductivity; low-pressure gasometry; electrical conductivity; infrared absorption; coulometry; and gravimetry.

Five laboratories used the coulometric method to produce one result on six separate days from each of a series of eight steels. The original report contains a statistical survey carried out to derive within- and between-laboratory standard deviations for each steel and method principle.

To test our coulometric method, we analysed samples of the same steels, and additional samples, once per day on ten different days, and calculated the means and standard deviations of the results. Results from the improved system are compared in Table II with those obtained[11] by the Carbon Study Group. It can be seen that our results are in every instance more reproducible than the typical results derived from the work of the Study Group, which had already demonstrated that coulometry and low-pressure gasometry were the most reproducible method principles of the seven principles tested.[2] The reproducibilities are also much better than those given by Thomich[8] for a range of steels analysed in quadruplicate in six German laboratories using the coulometric method for arbitrational analysis issued in 1971.[7]

On the other hand, it must be admitted that we have not yet reached our objective of attaining a long-term standard deviation of 0·001 per cent. of carbon at the 1 per cent. level, and further development work is in progress. This work is mainly devoted to reducing electrical noise and minute drifts in the high-impedance electronic circuits, to improving the electrode system and to speeding up the determination in order to minimise the effects of background noise and apparatus blanks.

The present operational cycle of 5 to 6 minutes makes the apparatus too slow for routine bath-sample analysis, but it is ideal for referee work and standardisation. It has already proved to be an extremely useful apparatus for the investigation of problems involving carbon heterogeneity in ingots and billets, in studies of case carburisation, and in measuring decarburisation caused in heat treatment. Its accuracy depends solely on electrochemical equivalents, the precision and stability of a high-grade calibrated resistor and a Weston cell.

Hence, it gives a completely independent check upon results obtained by the other traditional methods mentioned earlier.

TABLE II

CARBON RESULTS BY COULOMETRIC TITRATION

			Carbon, per cent.				
				B.I.S.R.A. group		D.H. coulometer	
Steel No.	Type	Amount of sample/g	Certificate or accepted value	Mean (six results) ($\bar{x}$)	Within laboratory standard deviation (σ_w)	Mean (ten results) ($\bar{x}$)	Standard deviation (σ)
B.C.S. 260/3	H.P. iron	1·0	$0{\cdot}001_5$	—	—	0·0017	0·0001
U.S.C. 11	—	1·0	—	0·0227	0·0005	$0{\cdot}0228_5$	$0{\cdot}0002_3$
B.C.S. 265/2	Mild steel	1·0	0·048	0·0491	0·0006	$0{\cdot}0493_0$	$0{\cdot}0002_2$
B.C.S. 333	18 Cr 8 Ni	1·0	0·066	0·0673	0·0008	$0{\cdot}0653_0$	$0{\cdot}0002_1$
B.C.S. 237/1	EN 32	1·0	0·105	0·1066	0·0009	0·1062	0·0005
B.S.C. (U.S.A.)	(Pins)	1·0	0·44–	—	—	0·4396	0·0009
DH 1	EN 8	0·2	0·385	—	—	0·3789	0·0014
B.C.S. 240/2	EN 8	0·2	0·41–	0·4136	0·0032	0·4106	0·0012
DH 2	EN 42	0·2	0·665	—	—	0·6659	0·0013
B.C.S. 220/1	7W 5 Mo	0·2	0·93–	0·9271	0·0064	0·9277	0·0015
DH 3	EN 44	0·2	1·005	—	—	$1{\cdot}006_6$	0·0011
B.C.S. 163/1	EN 44	0·2	1·21–	1·216	0·0055	$1{\cdot}209_4$	0·0016
DH 4	EN 44E	0·2	1·26–	—	—	$1{\cdot}269_5$	0·0027
B.C.S. 247/4	White iron	0·1	3·05	—	—	3·051	0·0034
B.C.S. 247/4	White iron	0·2	3·05	—	—	3·053	0·0041
B.C.S. 247/3	White iron	0·1	3·0	3·012	0·0146	—	—
Standardising count, measured at 29 to 34 °C			0·63358 at 30 °C	Observations corrected to 30 °C		$0{\cdot}6336_2$	$0{\cdot}0001_6$

The measuring circuits can readily be re-set to deal with other coulometric titrations, for example of sulphur dioxide or ammonia, and applications to the determination of sulphur, oxygen and nitrogen in steel are under consideration.

The authors are grateful to Mr. G. F. Smith, Technical Director, Dunford Hadfields Ltd., for permission to publish this paper. They also express their thanks to their colleagues, Mr. T. W. Lomas and Mr. M. Swift, who have been responsible for the design and construction of several versions of the electronic sections of the apparatus described.

REFERENCES

1. Boniface, H. J., and Jenkins, R. H., *Analyst*, 1971, **96**, 37.
2. Scholes, P. H., "Proceedings of the Twenty-Third B.I.S.R.A. Chemists' Conference," 1970, p. 37.
3. Abresch, K., and Claassen, I., *translated by* Leveson, L. L., "Coulometric Analysis," 1961, Chapman and Hall, London, 1965, pp. 154, 177 and 178.
4. *Metallurgia*, 1969, **79**, 265.
5. Büchel, E., "Internationales Jahrbuch Chemische Industrie," Vogt-Schild, Solothum, 1962–63.
6. "Handbuch für das Eisenhütten Laboratorium," Band 4, Schiedanalysen, Verein Deutscher Eisenhüttenleute, Düsseldorf, 1955, p. 73.
7. "Handbuch für das Eisenhütten Laboratorium," Band 5 (Supplement), Verein Deutscher Eisenhüttenleute, Düsseldorf, 1971.
8. Thomich, W., *Arch. EisenhüttWes.*, 1972, **43**, 239.
9. Abresch, K., and Lemm, H., *Arch. EisenhüttWes.*, 1959, **30**, 1.
10. Craig, D. N., and Hoffman, J. I., "Proceedings of the National Bureau of Standards Semicentenial Symposium on Electrochemical Standards," September 19th–21st, 1951, p. 13.
11. Bagshawe, B., and Scholes, P. H., Report CAC/122/73 of the Joint B.S.C./B.I.S.R.A. Chemical Analysis Committee, Determination of Carbon in Steel Study Group; Greenfield, A., and Scholes, P. H., Study Group Report MG/DA/400/70.

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A Highly Selective Direct Colorimetric Procedure for the Determination of Zirconium in Steel with Arsenazo III by Using a Pressure-digestion Technique for Sample Dissolution

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Zirconium in steel is determined colorimetrically with arsenazo III in 7·5 M perchloric acid solution. Chemically inert forms of zirconium, which remain unchanged after conventional treatment with acid, are dissolved by means of a pressure-digestion technique. Results obtained with six British Chemical Standard steel samples show excellent precision and accuracy. Interference studies with fifteen metals commonly found in steel indicate that the procedure is applicable to a wide range of steels. Concentrations of zirconium in excess of 0·001 per cent. *m/m* in steel samples can be determined.

Arsenazo III is an excellent colorimetric reagent for the determination of zirconium.[1] The arsenazo III - zirconium complex is formed at very high acid concentrations (*e.g.*, 9 M hydrochloric acid), at which concentrations most other metal complexes of arsenazo III are dissociated; hafnium(IV), thorium(IV), titanium(IV), lanthanum(III) and uranium(VI) are the only metals reported to interfere.[1] The sensitivity is high, the zirconium complex having a molar absorptivity of $1{\cdot}2 \times 10^5$ l mol^{-1} cm^{-1} under the conditions used by Dedkov, Ryabchikov and Savvin.[1]

A major difficulty in determining zirconium colorimetrically is caused by the extensive formation of unreactive polynuclear hydrolysis products by zirconium(IV).[2] Pakalns[3] has reported in detail on the depolymerisation of zirconium solutions prior to determining the zirconium with arsenazo III. Zirconyl chloride octahydrate solutions (1000 μg ml^{-1} of Zr) in 1 M perchloric acid, for example, were shown to react completely with arsenazo III, after being allowed to depolymerise at room temperature for 24 hours before use. Minimum concentrations of strong acids that are required in order to depolymerise zirconium solutions of several concentrations at their boiling-point and to maintain the zirconium in a monomeric condition, after cooling the solutions, are given. The results given for perchloric acid solutions have been used in this work in preparing standard zirconium solutions and have been found to give excellent results.

In a second paper, Pakalns[4] reported the determination of zirconium in steel with arsenazo III. His investigation of direct acid-dissolution procedures confirmed earlier work by Čechová[5] that separation of zirconium (with cupferron) was necessary when even small amounts of other metals that give precipitates due to hydrolysis were present; silica and other precipitates due to hydrolysis co-precipitate zirconium.

In the present work, a PTFE-lined steel pressure vessel was used to complete the acid dissolution of steel samples. A direct colorimetric finish with arsenazo III was then applied without prior separation of zirconium. Excellent results were obtained with this procedure, whereas low recoveries of zirconium were always obtained when dissolution was effected at atmospheric pressure alone. PTFE-lined pressure vessels have been used previously for the determination of trace elements in siliceous materials[6–8] and foodstuffs.[9,10] They have also been used in determinations of nitrogen[11] and total aluminium[12] in steel.

Experimental

If full advantage is to be taken of the fact that arsenazo III is a highly sensitive and selective reagent, then the colorimetric determination must be carried out in strongly acidic

media. As high concentrations of sulphate,[3] phosphate[3] and nitrate[13,14] interfere, the corresponding strong acids cannot be used. Kammori and co-workers[15] found that interference from nitrate could be overcome by the addition of urea; absorbance measurements were made in nitric acid solution, for which they claim that the absorbance is less dependent on the acidity of the solution. In the present work, this procedure was found to be unsatisfactory: the mean of six determinations at the 0·4 μg ml^{-1} level of zirconium gave an absorbance of 0·449 at 665 nm with a standard deviation of 0·039.

The choice of strong acid was therefore limited to hydrochloric and perchloric acids. With hydrochloric acid maximum absorbance occurs at 9 M concentration and with perchloric acid at 7·5 M concentration (see Fig. 1). Close to this maximum the acid concentration was slightly less critical with perchloric acid. Furthermore, 9 M hydrochloric acid is more concentrated than its azeotrope, which makes it difficult to use. For these reasons, perchloric acid was used as the reagent of choice.

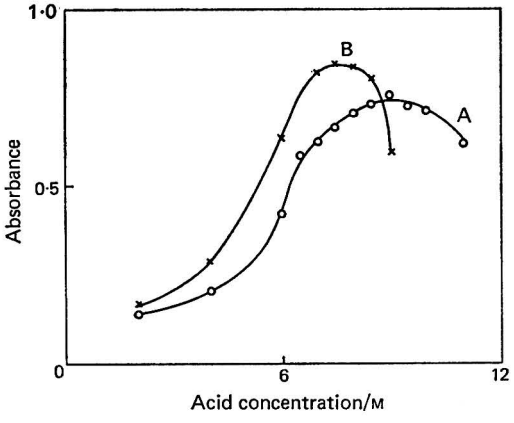

Fig. 1. Effect of hydrochloric and perchloric acid concentrations on the absorbance of the zirconium - arsenazo III complex: A, hydrochloric acid; and B, perchloric acid. Zirconium concentration 0·6 μg ml^{-1}; and arsenazo III concentration 0·002 per cent. *m/V*

The procedure for dissolution of steels by using a PTFE-lined pressure vessel is given in detail below. The results given in Table I indicate the necessity for the use of a pressure vessel. The inclusion of hydrofluoric acid in the dissolution mixture was found to be necessary even when the pressure vessel was used, and nitric acid was also included in the mixture in order to decompose carbides. The efficient removal of hydrofluoric and nitric acids by evaporation to fumes prior to colorimetric determination was found to be very important. Even low concentrations of fluoride interfere with the reaction of zirconium with arsenazo III.

By optimising reagent concentrations and volumes, and by examination of colour development and stability times, the procedure outlined below was developed and is recommended for general use. Ascorbic acid was added to sample solutions at the colorimetric stage in order to mask iron(III); it was not necessary to add it to standard solutions in the preparation of the calibration graph. The pressure-digestion vessel used in this work was obtained from S. and J. Juniper and Co., Harlow, Essex.

REAGENTS—

Aqua regia—Mix equal volumes of concentrated hydrochloric acid (analytical-reagent grade, sp. gr. 1·16 to 1·18) and concentrated nitric acid (analytical-reagent grade, sp. gr. 1·42).

Hydrofluoric acid, 40 per cent. m/V—Analytical-reagent grade.

Perchloric acid, 7·5 M—Dilute 1300 ml of analytical-reagent grade concentrated perchloric acid (70 per cent. *m/V*) to 2 litres in a calibrated flask.

Ascorbic acid—Analytical-reagent grade.

Arsenazo III solution, 0·1 per cent. m/V—Dissolve 0·1 g of arsenazo III plus about 0·5 g of sodium hydroxide in about 50 ml of water. Add concentrated hydrochloric acid dropwise with stirring, until the colour of the solution just changes to red - violet, and dilute the solution to 100 ml with water in a calibrated flask.

Concentrated standard zirconium solution in 1 M *perchloric acid, 100 μg ml⁻¹ of Zr*—Dissolve 0·177 g of zirconyl chloride octahydrate in about 200 ml of 1 M perchloric acid by boiling the mixture under reflux for 1 hour. Cool and dilute the solution to 500 ml with 1 M perchloric acid in a calibrated flask.

Dilute standard zirconium solution in 7·5 M *perchloric acid, 10 μg ml⁻¹ of Zr*—By pipette, transfer 10 ml of concentrated standard zirconium solution into a 100-ml calibrated flask, add 62 ml of 70 per cent. *m/V* perchloric acid solution and dilute to 100 ml with water.

TABLE I

RECOVERIES OF ZIRCONIUM FROM B.C.S. STEEL 271 BY USING DIFFERENT DISSOLUTION PROCEDURES

Dissolution procedure	Zirconium found, per cent. *m/m*	Coefficient of variation,* per cent.	Recovery,† per cent.
Boiling 7·5 M $HClO_4$ for 30 minutes	0·0033	7	7·5
Preliminary dissolution in HCl and HF, followed by evaporation to fumes with 7·5 M $HClO_4$ so as to drive off excess of HF ..	0·0395	6	88
Recommended procedure with pressure digestion vessel	0·044	2	100

* Six determinations.
† Based on results given in Table III.

PREPARATION OF CALIBRATION GRAPH—

By pipette, introduce aliquots of the dilute standard zirconium solution (0 to 3·0 ml) into dry 50-ml calibrated flasks. To each flask add, by pipette, 1 ml of arsenazo III solution and dilute the mixture to 50 ml with 7·5 M perchloric acid. Measure the absorbance of the solution at 664 nm within 15 minutes of preparation against water, using 1-cm cells. Subtract the absorbance of the solution that contains no zirconium.

PROCEDURE—

Remove the PTFE liner from the pressure vessel and weigh into the liner an appropriate amount of steel (Note 1 and Table II). Add 20 ml of aqua regia and allow the steel sample to dissolve without heating. When evolution of hydrogen has ceased, add 5 ml of 40 per cent. *m/V* hydrofluoric acid solution, place and seal the liner in the pressure vessel (Note 2 and instruction manual where appropriate) and leave the pressure vessel in an oven at 200 °C overnight (Note 3).

TABLE II

SUGGESTED SAMPLE SIZES AND ALIQUOTS FOR USE WITH VARIOUS STEELS

Expected zirconium content, per cent. *m/m*	Amount of steel to be taken/g	Aliquot taken/ml
0·001 to 0·006	0·5	25
0·006 to 0·01	0·5	15
0·01 to 0·02	0·5	10
0·02 to 0·04	0·5	5
0·04 to 0·05	0·1	15
0·05 to 0·10	0·1	10
0·10 to 0·20	0·1	5

Cool the pressure vessel to room temperature and open it carefully. Transfer the contents quantitatively into a suitable PTFE vessel with 25 ml of 7·5 M perchloric acid. Reduce the volume to less than 10 ml by heating the vessel on a hot-plate (Note 4), add 25 ml of 7·5 M perchloric acid and warm the mixture, if necessary, to dissolve any crystalline material (Note 5). Transfer the solution into a 50-ml calibrated flask and dilute it to 50 ml with 7·5 M perchloric acid.

With a pipette, transfer an appropriate aliquot of the solution (see Table II) into a second 50-ml calibrated flask, add 1 g of ascorbic acid, dilute to about 30 ml with 7·5 M perchloric

acid and swirl the flask to dissolve the ascorbic acid without heating. Continue the determination as described under Preparation of calibration graph beginning at "add, by pipette, 1 ml of arsenazo III solution. . . ." For steels containing large amounts of metals that give coloured ions, determine the absorbance at 664 nm of an aliquot of steel solution in the absence of arsenazo III and deduct this absorbance together with the arsenazo III reagent blank from the absorbance value obtained with the sample.

Although in many instances the zirconium content of the steel will not be known even approximately, the sample sizes and aliquots recommended in Table II will give an indication of a suitable amount of sample to take as a trial. The sample size and aliquots recommended for each range of zirconium content give absorbance values between 0·3 and 0·6, except for steel samples containing less than 0·004 per cent. *m/m* of zirconium.

NOTES—

1. *Caution*—Perchloric acid must NOT be included in the digestion mixture in the sealed pressure vessel. The liner should be perfectly clean and dry, and care should be taken to ensure that no traces of perchloric acid from the previous determination remain in the liner.

2. Corrosion products tend to form at the surfaces between the metal and the liner. These surfaces should be cleaned regularly in order to avoid difficulties arising in removing the liner after digestion.

3. For some steels this digestion period can be reduced. Complete recovery of zirconium was made from British Chemical Standards Steels Nos. 271, 272, 274 and 275 after only 2 hours' digestion.

4. This evaporation stage to remove hydrogen fluoride and oxides of nitrogen, although time consuming, is very important. Trace amounts of fluoride, in particular, interfere with the reaction of zirconium with arsenazo III.

5. With steels that have a high chromium content, a red chromium(VI) compound may crystallise out on evaporating the solution to 10 ml. This precipitate dissolves more readily if about 1 g of ascorbic acid is added after the 7·5 M perchloric acid, which treatment should be carried out *without warming* as ascorbic acid is rapidly decomposed by warm perchloric acid solutions.

Results obtained with the procedure described for the preparation of the calibration graph with the dilute standard zirconium solution had a coefficient of variation of 1·5 per cent. for ten determinations at the 0·4 μg ml^{-1} of zirconium level. The calibration graph was linear in the range 0·1 to 0·6 μg ml^{-1} of zirconium, and its slope corresponded to a molar absorptivity for the complex of $1{\cdot}32 \times 10^5$ l mol^{-1} cm^{-1} at 664 nm. Results obtained with six British Chemical Standards steel samples by using the recommended procedure are given in Table III. These results show good reproducibility and compare well with those obtained by other methods.

TABLE III

DETERMINATION OF ZIRCONIUM IN BRITISH CHEMICAL STANDARD STEELS

	Zirconium content, per cent. *m/m*				
	B.C.S. values*		Xylenol† orange procedure	X-ray‡ fluorescence	Recommended procedure§
Steel No.	Chemical	Spectrographic			
271	$0{\cdot}04_5$	$0{\cdot}04_5$	0·044	0·043	0·044, 0·045, 0·043
272	$0{\cdot}03_0$	$0{\cdot}03_0$	0·031	0·029	0·031, 0·033, 0·033
274	$0{\cdot}00_5$	$0{\cdot}01_0$	0·012	0·010	0·010, 0·011, 0·011
275	$0{\cdot}01_5$	$0{\cdot}02_0$	0·021	0·019	0·020, 0·020, 0·021
276	$0{\cdot}00_5$	$0{\cdot}00_5$	0·008	0·006	0·006, 0·006, 0·005
277	$0{\cdot}04_0$	$0{\cdot}05_0$	0·051	0·050	0·050, 0·048, 0·047

* Zirconium is a non-standardised element in these steels.
† Results of Keller and Hennesen.[16]
‡ Results of Klima and Scholes.[17]
§ Results obtained from three different dissolutions of the steel sample.

INTERFERENCES—

The effects of fourteen other metals on the determination of zirconium are shown in Table IV. The metal to zirconium ratios chosen for study are well above those normally

found in steels. Of these metals only titanium interferes and then only when present above about a thirty-fold ratio of titanium to zirconium.

TABLE IV

INTERFERENCE RESULTS

Amount of zirconium added 20·0 μg

Metal	Metal to zirconium ratio	Zirconium found/μg
Antimony	10	19·3, 19·2
Arsenic	100	19·6, 19·5
Manganese	10 000	20·0, 20·2
Copper	10 000	20·7, 20·9*
Titanium	30	19·3, 19·3
	50	18·8, 19·0
	100	17·5, 17·9
	300	11·3, 9·3
Aluminium	1000	19·8, 19·2
Tin	100	19·8, 19·0
Lead	100	20·3, 19·5
Beryllium	1000	20·5, 19·5
Molybdenum	1000	19·3, 19·3
Chromium	10 000	18·8, 19·3*
Vanadium	1000	19·5, 19·3*
Cobalt	10 000	19·3, 19·0
Nickel	3000	19·3, 19·0*

* These metals produced ions in the dissolution process, which absorbed at 664 nm. A correction was made for this effect (see text).

Tungsten, which has not been included in Table IV, is the only metal that interferes seriously. A colloidal precipitate of yellow tungstic acid forms when the digestion mixture is evaporated to 10 ml. This precipitate cannot readily be filtered or centrifuged, and it is probable that zirconium is co-precipitated. Consequently, the procedure in its present form cannot be applied to high-tungsten steels. Of the steels studied here, B.C.S. 276 and 277 contained the largest amount of tungsten (0·20 and 0·12 per cent. *m/m*, respectively), and this amount of tungsten did not interfere in the determination.

DISCUSSION

The procedure given above is recommended as it gives highly satisfactory results. No prior separation of zirconium is required owing to the use of a PTFE-lined pressure-digestion vessel that eliminates most interferences. Pakalns[4] has suggested that the low results obtained with conventional acid-dissolution procedures are caused by co-precipitation of zirconium with silica and other precipitates that are due to hydrolysis. In the present work, the necessity for an intense pressure treatment of the steel sample with a digestion mixture containing hydrofluoric acid in order to release the zirconium has been demonstrated. It seems more probable to the present authors that the intense treatment is required so as to dissolve refractory zirconium compounds present in the steel rather than to avoid co-precipitation.

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REFERENCES

1. Dedkov, Yu. M., Ryabchikov, D. I., and Savvin, S. B., *Zh. Analit. Khim.*, 1965, **20**, 574.
2. Blumenthal, W. B., *Talanta*, 1968, **15**, 877.
3. Pakalns, P., *Analytica Chim. Acta*, 1969, **44**, 73.
4. ——, *Ibid.*, 1971, **57**, 51.
5. Čechová, D., *Chemist Analyst*, 1967, **56**, 94.
6. Bernas, B., *Analyt. Chem.*, 1968, **40**, 1682.
7. Schnetzler, C. C., and Nava, D. F., *Earth Planetary Sci. Lett.*, 1971, **11**, 345.
8. Nava, D. F., Walter, L. S., and Doan, A. S., jun., *J. Geophys. Res.*, 1971, **76**, 4067.

9. Nelson, G., and Smith, D. C., *Proc. Soc. Analyt. Chem.*, 1972, **9**, 168.
10. Holack, W., Krinitz, B., and Williams, J. C., *J. Ass. Off. Analyt. Chem.*, 1972, **55**, 741.
11. Menis, O., N.B.S. Technical Note 454, National Bureau of Standards, Washington, D.C., 1968.
12. Headridge, J. B., and Sowerbutts, A., *Analyst*, 1973, **98**, 57.
13. Savvin, S. B., *Talanta*, 1961, **8**, 673.
14. Savvin, S. B., Kadaner, D. S., and Ryabova, A. S., *Zh. Analit. Khim.*, 1964, **19**, 561.
15. Kammori, O., Taguchi, I., and Komiya, R., *Bunseki Kagaku*, 1965, **14**, 106.
16. Keller, H., and Hennesen, K., *Arch. EisenhüttWes*, 1968, **39**, 921.
17. Klima, Z., and Scholes, P. H., *Analyst*, 1973, **98**, 351.

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Determination of an Isomeric Impurity in Samples of Morantel Tartrate by Gas - Liquid Chromatographic Analysis of the Products of a Controlled Degradation Procedure

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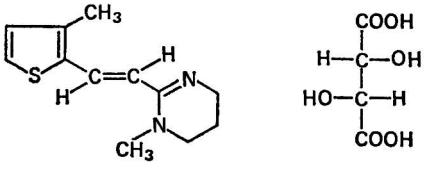
A procedure is described for the determination of the tartrate salt of the 4-methylthienyl isomer of morantel, which is present in samples of morantel tartrate. The procedure involves a controlled degradative oxidation of the ethylene bond that converts morantel and the 4-methylthienyl isomer of morantel into 3-methylthiophene-2-carbaldehyde and 4-methylthiophene-2-carbaldehyde, respectively. Extraction of the two aldehydes is followed by the determination of their relative concentrations by a gas - liquid chromatographic procedure. It is shown that the ratio of the aldehydes given by gas - liquid chromatography is not significantly different from the ratio of morantel to the 4-methylthienyl isomer of morantel present in prepared mixtures of the two isomers. The method is shown to have good precision and to be applicable to morantel samples containing 0·10 to 7·00 per cent. *m/m* of the 4-methylthienyl impurity.

MORANTEL tartrate is a broad-spectrum anthelmintic agent used for the treatment of a wide variety of infestations in domestic animals. The chemical structure of the compound is given below (**I**).

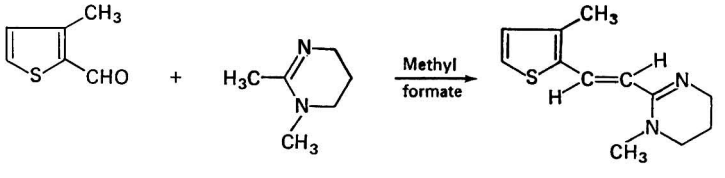


Morantel tartrate ($C_{16}H_{22}N_2O_6S$)

I

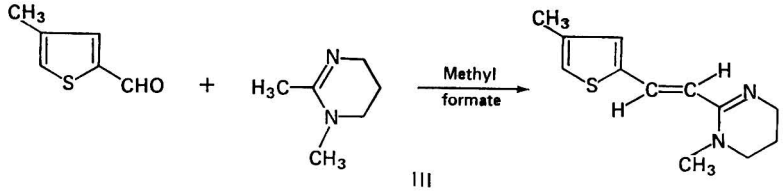
The chemical name of morantel tartrate is 1,4,5,6-tetrahydro-1-methyl-2-[*trans*-2-(3-methyl-2-thienyl)vinyl]pyrimidine hydrogen tartrate.

The synthesis of morantel involves the condensation of 3-methylthiophene-2-carbaldehyde with 1,4,5,6-tetrahydro-1,2-dimethylpyrimidine in the presence of methyl formate, as shown in **II**.



II **Morantel**

The 3-methylthiophene-2-carbaldehyde used in this step always contains an appreciable proportion (approximately 11 per cent. *m/m*) of the isomeric impurity 4-methylthiophene-2-carbaldehyde. It is not possible to remove the impurity at this stage and hence, during this reaction sequence, it reacts with 1,4,5,6-tetrahydro-1,2-dimethylpyrimidine according to the scheme shown (**III**) to give 1,4,5,6-tetrahydro-1-methyl-2-[*trans*-2-(4-methyl-2-thienyl)-vinyl]pyrimidine, which is referred to as the 4-methylthienyl isomer.



III

Crystallisation of crude morantel tartrate reduces the level of the 4-methylthienyl isomer considerably, but it is necessary quantitatively to monitor the amount of this isomeric impurity present in the finished product. The method described in this paper will determine the 4-methylthienyl isomer down to a level of 0·10 per cent. *m/m*.

Morantel and the 4-methylthienyl isomer of morantel have very similar physical and chemical properties, governed largely by the polar, highly basic, tetrahydropyrimidine ring. In order to accentuate the difference in molecular structure for the purposes of chromatographic separation, a degradation procedure has been devised that oxidises the ethylenic bond[1] connecting the thiophene and tetrahydropyrimidine ring systems. This selective oxidation of morantel and its 4-methylthienyl isomer produces corresponding amounts of 3-methylthiophene-2-carbaldehyde and 4-methylthiophene-2-carbaldehyde. The use of alkaline permanganate solution in conjunction with rapid extraction of the carbaldehydes into toluene avoids over-oxidation of the ethylenic bonds to the corresponding carboxylic acids. The ratio of the two carbaldehydes produced is determined by a gas - liquid chromatographic procedure.

It is essential to verify the absence of these two carbaldehydes in samples before carrying out the determination.

Experimental and results

Reagents and materials—

Anhydrous sodium carbonate—AnalaR grade.

Potassium permanganate, 1·00 per cent. m/V aqueous solution—Dissolve AnalaR grade potassium permanganate (1·00 ± 0·01 g) in distilled water and make the volume up to 100 ml. Potassium permanganate solutions are not stable indefinitely; the solution should therefore be stored in a dark bottle and replaced 3 days after preparation.

Toluene—AnalaR grade.

Gas - liquid chromatographic column packing—Gas-Chrom Q (100 to 120 mesh), coated with 5 per cent. *m/m* polyethylene glycol 400, is a suitable packing and can be prepared in the laboratory or purchased from Perkin-Elmer Ltd., Beaconsfield, Bucks.

Apparatus—

Separating funnel, 50-ml capacity.

Safety pipettes, 2-ml and 10-ml capacity.

Phase-separating filter-papers, Type 1 PS, of 9·0 cm diameter (Whatman Ltd.).

Gas - liquid chromatograph equipped with a flame-ionisation detector.

Glass column for gas - liquid chromatography—The column should be 3·0 m long, with an internal diameter of 1·5 mm. The configuration of the column is dependent on the model of gas chromatograph used. For the Varian, Model 204, gas chromatograph used by us, the column has a helical configuration.

Potentiometric recorder with a range of 2·5 mV.

Syringe for gas - liquid chromatography, capable of injecting 1·0 *μl liquid samples.*

Graticule, 25 mm with divisions every 0·1 *mm*—This was a Type T1 from Graticules Ltd., 18–20 Garrick Street, London, W.C.2.

CHROMATOGRAPHIC CONDITIONS—

The following conditions are used: glass column, 3·0 m by 1·5 mm (the column used in this laboratory had an outside diameter of $\frac{1}{8}$ inch); packing, Gas-Chrom Q (100 to 120 mesh) coated with 5 per cent. *m/m* polyethylene glycol 400; column temperature, 105 °C isothermal (see NOTE); injection temperature, 150 °C; detector temperature, 150 °C; carrier gas, nitrogen at a flow-rate of 30 ml min^{-1}, measured at room temperature (see NOTE); and sample size, 1·0 μl.

Under these conditions, the two components of interest had the following retention times from injection: 4-methylthiophene-2-carbaldehyde, 29·0 minutes; and 3-methylthiophene-2-carbaldehyde, 31·8 minutes (separation factor = 1·10).

In order to facilitate accurate measurement of peak areas, a column is required that has approximately 4000 theoretical plates.[2] The work described here was carried out on a column with 5000 theoretical plates, measured by using the 3-methylthiophene-2-carbaldehyde peak.

NOTE—The temperature of the column and flow-rate of the carrier gas may require adjustment when different chromatographs and different column configurations are used.

PROCEDURE—

Add the morantel tartrate sample (0·10 ± 0·01 g) to 10 ml of distilled water. Ensure that dissolution is complete and then transfer the solution to a 50-ml separating funnel. Add 1·0 g of sodium carbonate to the separating funnel and shake the funnel until the carbonate is dissolved. Add 10 ml of the potassium permanganate solution to the separating funnel with a pipette over a period of 1 minute while swirling the contents and allow the mixture to stand for 2 minutes before adding toluene (2·0 ml) to the funnel, again by means of a pipette. Extract the aldehydes into the toluene by shaking the separating funnel for 2 minutes, then discard the aqueous phase and transfer the organic phase to a small vial by filtering it through a phase-separating filter-paper. Inject 1·0 μl of the toluene solution

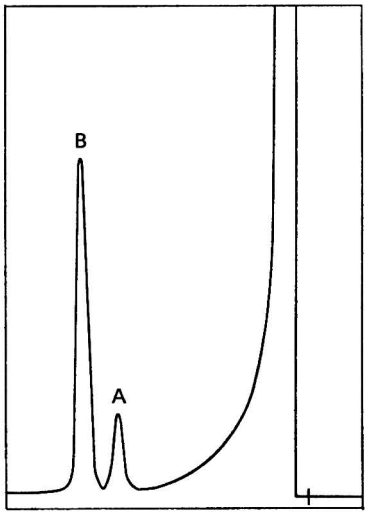


Fig. 1. Gas - liquid chromatogram showing the separation of (4-methylthiophene-2-carbaldehyde (A) from 3-methylthiophene-2-carbaldehyde (B). Chromatographic conditions are given in the text

on to the gas - liquid chromatographic column and record the chromatographic peaks due to the 4-methylthiophene-2-carbaldehyde and 3-methylthiophene-2-carbaldehyde at suitable attenuation settings. Measure the peak heights to the nearest 0·5 mm and peak widths to the nearest 0·1 mm by using a ruler and the graticule, respectively. Calculate the area of each peak by multiplying the peak height by the width at half-height. A typical chromatogram is shown in Fig. 1.

CALCULATION—

Let A represent the area of the 4-methylthiophene-2-carbaldehyde peak, B the area of the 3-methylthiophene-2-carbaldehyde peak, a the attenuation of the 4-methylthiophene-2-carbaldehyde peak and b the attenuation of the 3-methylthiophene-2-carbaldehyde peak. Then, the percentage m/m of the 4-methylthienyl isomer in the sample of morantel tartrate is $Aa/(Aa + Bb) \times 100$. For examples of this calculation, see Table I.

TABLE I

ANALYSES OF A SYNTHETIC BLEND OF MORANTEL TARTRATE AND THE TARTRATE SALT OF THE 4-METHYLTHIENYL ISOMER

Ten replicate analyses

Run	Peak height of 4-isomer*/ mm (attenuation × 1)	Peak width at half-height of 4-isomer*/ mm	Peak height of 3-isomer*/ mm (attenuation × 16)	Peak width at half-height of 3-isomer*/ mm	Area of 4-isomer* peak/mm² (attenuation × 1)	Area of 3-isomer* peak/mm² (attenuation × 16)	Content of 4-isomer, per cent. m/m
A	69·5	5·0	178·0	5·0	347·5	890	2·38
B	82·0	4·8	214·0	5·0	393·6	1070	2·25
C	70·5	4·7	185·0	5·0	331·4	925	2·19
D	82·5	4·9	218·0	5·1	404·3	1112	2·22
E	78·5	4·8	215·0	5·2	376·8	1118	2·06
F	80·0	4·9	212·0	5·1	392·0	1081	2·22
G	86·0	4·8	227·0	5·1	412·8	1158	2·18
H	90·0	4·8	225·5	5·1	432·0	1150	2·29
J	70·5	4·9	189·0	5·1	345·5	964	2·19
K	62·0	4·7	159·5	5·0	291·4	798	2·23

* The abbreviations 3-isomer and 4-isomer refer to 3-methylthiophene-2-carbaldehyde and 4-methylthiophene-2-carbaldehyde, respectively.

Using the above results, standard statistical calculations give: mean value = 2·221; variance = $[S_{(10)}]^2$ = 0·0067; standard deviation = $S_{(10)}$ = 0·082.

BLANK DETERMINATION—

It is essential to verify the absence of the two carbaldehydes in samples analysed by the above method. This verification can be conveniently accomplished by use of a blank determination.

Follow the described procedure with a second sample of morantel tartrate but omit the addition of potassium permanganate solution. The gas - liquid chromatographic examination of the toluene extract must show no evidence of either carbaldehyde.

PRECISION—

In order to evaluate the precision of the method, a solution was prepared that contained approximately 1·0 g of morantel tartrate and 0·02 g of the 4-methylthienyl isomer per 100 ml of distilled water, corresponding to an impurity level of approximately 2·0 per cent. m/m of the 4-methylthienyl isomer with respect to morantel. This level was chosen because of its relevance to quality control limits.

Ten replicate analyses were carried out on 10-ml aliquots of the solution by following the given procedure. The results and calculations are given in Table I. The standard deviation calculated from the results, $S_{(10)}$, is 0·082. Thus, the result of a single determination can be quoted as being $\pm$0·19 per cent. m/m with 95 per cent. confidence.

In order to evaluate the error involved in the gas - liquid chromatographic determination of the aldehydes, a toluene solution containing 4-methylthiophene-2-carbaldehyde and

3-methylthiophene-2-carbaldehyde was chromatographed seven times. The relative amounts of the two isomers were calculated by the method given under Prodecure and Calculation. The standard deviation for the seven replicates was calculated to give a value for $S_{(7)}$ of 0·057 per cent. *m/m*. Results are given in Table II.

TABLE II

RESULTS OF GAS - LIQUID CHROMATOGRAPHY RUNS ON THE SAME TOLUENE SOLUTION OF 3-METHYLTHIOPHENE-2-CARBALDEHYDE AND 4-METHYLTHIOPHENE-2-CARBALDEHYDE

Seven replicate analyses

Run	Peak height of 4-isomer*/ mm (attenuation × 1)	Peak width at half-height of 4-isomer*/ mm	Peak height of 3-isomer*/ mm (attenuation × 16)	Peak width at half-height of 3-isomer*/ mm	Area of 4-isomer* peak/mm² (attenuation × 1)	Area of 3-isomer* peak/mm² (attenuation × 16)	Content of 4-isomer, per cent. *m/m*
1	73·0	4·8	180·5	5·1	350·4	921	2·32
2	74·0	4·9	177·5	5·1	362·6	905	2·44
3	66·5	4·7	162·5	5·1	312·6	829	2·30
4	73·0	4·7	180·0	5·0	343·1	900	2·33
5	69·5	4·8	173·5	5·1	333·6	885	2·29
6	70·0	4·7	174·0	5·0	329·0	870	2·30
7	79·0	4·7	188·5	5·0	371·3	943	2·40

* The abbreviations 3-isomer and 4-isomer refer to 3-methylthiophene-2-carbaldehyde and 4-methylthiophene-2-carbaldehyde, respectively.

Using the above results, standard statistical calculations give: mean value = 2·341; $[S_{(7)}]^2$ = 0·0032; standard deviation $S_{(7)}$ = 0·057.

Application of the *F* test shows no significant difference between the variance for the gas - liquid chromatographic analysis and the variance associated with the whole procedure. Therefore, if increased precision is required it is advisble to replicate the gas - liquid chromatographic analysis. As the standard deviation for the whole procedure, $S_{(10)}$ is 0·082, the 95 per cent. confidence limit for the mean of n replicates is given by the term $0{\cdot}19n^{-\frac{1}{2}}$ at the 2 per cent. *m/m* level.

ACCURACY—

The accuracy of the procedure was evaluated by making standard additions of the tartrate salt of the 4-methylthienyl isomer to a sample of morantel tartrate, followed by analysis of the resulting mixtures.

A standard solution of morantel tartrate was prepared by weighing 0·9761 g into a 100-ml calibrated flask, dissolving it in, and making the solution up to volume with, distilled water. This is referred to as solution A. A standard solution of the 4-methylthienyl isomer was prepared by weighing 0·1241 g into a 100-ml calibrated flask, again dissolving it in, and making the solution up to volume with, distilled water. This is referred to as solution B.

Aliquots of solution A (10·0 ml) were transferred by pipette into 50-ml separating funnels together with various aliquots (0, 1·0, 2·0, 3·0, 4·0, 5·0 and 6·0 ml) of solution B. The resulting solutions thus contained known amounts of morantel and its 4-methylthienyl isomer. The solutions were then taken through the remainder of the procedure, commencing at "Add 1·0 g of sodium carbonate. . . ." The results are presented in Table III.

The results show that the accuracy of the method is acceptable. The graph of the percentage (*m/m*) of impurity added *versus* the percentage (*m/m*) of the impurity found is linear and has a slope close to unity, proving that the ratio of 4-methylthiophene-2-carbaldehyde to 3-methylthiophene-2-carbaldehyde produced in the oxidation step is consistent with the ratio of the isomeric 4-methylthienyl impurity to morantel present in samples of morantel tartrate. The fact that the line diverges slightly from a slope of unity is indicative of a small bias in the method. The source of this bias is not known but it is small in comparison with the precision of the method. Thus, at a 4-methylthienyl isomer content of 2·00

per cent. *m/m*, the bias is 0·08 per cent. *m/m*, whereas the precision of the determination is ±0·19 per cent. *m/m* with a 95 per cent. confidence limit.

TABLE III

ANALYSES OF SYNTHETIC BLENDS OF MORANTEL TARTRATE AND THE TARTRATE SALT OF THE 4-METHYLTHIENYL ISOMER OF MORANTEL

Single analyses

Run	Solution A/ml	Solution B/ml	4-isomer* added, per cent. *m/m*	Peak height of 4-isomer*/mm (attenuation)	Peak width at half-height of 4-isomer*/mm	Peak height of 3-isomer*/mm (attenuation)	Peak width at half-height of 3-isomer*/mm	Area of 4-isomer* peak/mm²	Area of 3-isomer* peak/mm²	Content of 4-isomer*, per cent. *m/m*
1	10	0	0	—	—	—	—	Nil	50 094	Not detected
2	10	1·0	1·26	69·5 (A2)	4·5	163 (A64)	4·8	626	50 074	1·23
3	10	2·0	2·48	132 (A2)	4·3	157 (A64)	4·7	1125	47 226	2·33
4	10	3·0	3·67	202·5 (A2)	4·3	159 (A64)	4·7	1742	47 827	3·51
5	10	4·0	4·84	122·5 (A4)	4·3	143·5 (A64)	4·7	2107	43 165	4·65
6	10	5·0	5·98	149 (A4)	4·3	141·5 (A64)	4·6	2563	41 658	5·80
7	10	6·0	7·09	182 (A4)	4·3	142 (A64)	4·7	3130	42 714	6·83

* The abbreviations 3-isomer and 4-isomer refer to 3-methylthiophene-2-carbaldehyde and 4-methylthiophene-2-carbaldehyde, respectively.

In addition, regression analysis of the results in Table III shows that the intercept is not significantly different from zero. In view of the peak heights given in this table, the demonstrated linearity of the results and the lack of any response when nil 4-methyl isomer is added, the stated lower limit for this method of 0·1 per cent. *m/m* is a conservative estimate.

It is concluded that the method is adequate for the quantitative determination of 1,4,5,6-tetrahydro-1-methyl-2-[*trans*-2-(4-methyl-2-thienyl)vinyl]pyrimidine hydrogen tartrate, an isomeric impurity present in samples of morantel tartrate. The method is rapid, easily carried out, makes use of readily available laboratory apparatus and can be used for quality control purposes.

REFERENCES

1. Migrdichian, V., "Organic Synthesis," Volume 2, Reinhold Publishing Corporation, New York, 1957, p. 896.
2. Keulemans, A. I. M., "Gas Chromatography," Reinhold Publishing Corporation, New York, 1959.

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Determination of Thiabendazole in Citrus Fruits by Ultraviolet Spectrophotometry*

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A method for the ultraviolet spectrophotometric determination of thiabendazole in citrus fruit is described. It is extracted from the fruits by equilibrating the mashed peels or pulps with chloroform, the extract is acidified and the mixture concentrated in order to eliminate the chloroform and filtered. After making the filtrate slightly alkaline the thiabendazole is re-extracted with chloroform and determined by measuring the absorption of the extract at 302 to 303 nm. The procedure enables the satisfactory elimination of interfering substances to be achieved, and small amounts of thiabendazole of the order of 0·1 p.p.m. in the peel and the pulps and 0·03 p.p.m. in the whole fruit to be determined. The recovery of thiabendazole added to the peel or pulp of fruit varies between 94·1 and 103·0 per cent.

Thiabendazole [2-(4′-thiazolyl)benzimidazole, $C_{10}H_7N_3S$], has been used for some time as a fungicide for the post-harvest treatment of citrus fruits so as to reduce the incidence of rot in stored fruits.[1] There is a need for a routine inexpensive method for determining minute amounts of thiabendazole, on or in the citrus fruits (often less than 1 p.p.m. in the whole fruit). In some of the procedures previously described, it is extracted from citrus fruit with ethyl acetate[2–4] and, after elimination of the interfering substances, determined in hydrochloric acid solution by spectrofluorimetry[2] or by ultraviolet spectrophotometry,[3] in methanolic solution by spectrofluorimetry[4] and in ethyl acetate solution by gas chromatography[3]; in other procedures, the thiabendazole is extracted from the fruit with dichloromethane and determined either colorimetrically[5] or in methanolic solution by ultraviolet spectrophotometry.[6]

Whichever method is used, the main problems in its determination in citrus fruits occur in its quantitative extraction from the fruit and in the elimination of interfering substances. Ethyl acetate is used for the extraction of thiabendazole in most methods because of the very slight solubility of this compound in most other organic solvents.[3]

The extractives interfering in the determination are eliminated by subjecting the ethyl acetate extract to liquid - liquid extractions.[2–4] Those which interfere in the fluorimetric determination are not always completely removed[4] and the liquid - liquid extraction is combined with the separation of thiabendazole by thin-layer chromatography.[4] The recovery of thiabendazole added to whole-fruit blends is about 85 per cent.[4] or 90 per cent.[3] and is lower than when it is added to surface-stripping solutions of intact fruits.[4] In the method described herein, thiabendazole is extracted from the fruit with chloroform and determined in the chloroform solution by ultraviolet spectrophotometry. The procedure described for its extraction and for the clean-up of interfering substances is based on the solubility of thiabendazole in various solvents and on its distribution behaviour between chloroform and various aqueous solutions. The procedure is simple, inexpensive and enables the determination of thiabendazole in the fruits to be made with satisfactory accuracy.

Method

Apparatus—

All glassware must be scrupulously clean and all joints made of ground glass.

Ultraviolet spectrophotometer—A Perkin-Elmer spectrophotometer, Model 137, and stoppered rectangular silica cells, of 10 and 40-mm path length, were used.

Blenders—A Vir-Tis "45" homogeniser with a 500-ml stainless-steel container, and a Waring-type blender with a 1000-ml jar, were used.

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Concentration apparatus—Flat-bottomed 350-ml flasks with short necks with ground-glass stoppers, a Vigreux reflux column of effective length 15 cm, a condenser and a connection tube.

Separating funnels, 100 and 500-ml capacity.

Microburette, 2-ml capacity, with 0·01-ml divisions.

REAGENTS—

All reagents should be of recognised analytical grade.

Thiabendazole—As supplied by Merck, Sharp & Dohme.

Chloroform.

Sodium hydrogen carbonate solution, 5 per cent. m/V.

Hydrochloric acid, 0·1 N.

Sodium hydroxide solution, 1 N.

Trisodium citrate dihydrate.

Sodium sulphate, anhydrous.

pH indicator papers—Whatman - BDH indicator papers, narrow range pH 4 to 6, 8 to 10, and 10 to 12, were used.

ADJUSTMENT OF pH—

A 2-ml volume of 1N sodium hydroxide solution added with a microburette to 20 ml of 0·1N hydrochloric acid gives a mixture with a pH between 10·5 and 11, which is determined with a pH meter. Addition of 0·5 ml of the 5 per cent. sodium hydrogen carbonate solution depresses the pH of the mixture to about 9·5 which is also determined with a pH meter. In the procedure described below the required pH is checked with pH indicator paper and this check can be achieved by placing on the paper a drop of the above solution before and after addition of the sodium hydrogen carbonate and noting the colour and the nature of the developed stains corresponding to pH 10·5 to 11 and 9·5, and making the necessary comparisons.

GENERAL PROCEDURE

Thiabendazole, which is localised mainly in the peel of the fruit, is determined separately in the peel and in the pulp and calculated, as necessary, for the whole fruit. It can be determined directly in the whole fruit by following the procedure described for the pulp. It is extracted from mashed peel or pulp by equilibration[7] of the latter with chloroform; the chloroform extract is concentrated and hydrochloric acid is added to the concentrate, which is then concentrated, filtered and the filtrate made slightly alkaline. The thiabendazole is extracted from this solution with chloroform and transferred into 0·1 N hydrochloric acid solution. After making the latter slightly alkaline, the thiabendazole is re-extracted with chloroform and determined by measuring the absorption of the extract at 302 to 303 nm.

PREPARATION OF FRUIT EXTRACTS—

Preparation of peel samples—Weigh an average sample of fruit (for example, ten fruits) and remove carefully the peel including the albedo so as to avoid contaminating the pulp with trace amounts of thiabendazole from the peel. During peeling, prepare an average sample of peel by setting aside from each fruit an average sample of peel corresponding to about one third of the fruit surface. Chop the peel on a plate, mix it and take for analysis an accurately weighed amount not exceeding 100 g.

Preparation of pulp samples—Weigh the pulps and calculate the total amount of peel. By taking an aliquot from each pulp prepare a mixed sample of pulp not exceeding 500 g. Weigh the sample accurately and place it in the 1000-ml jar of the Waring blender. For each 100 g of orange or grapefruit pulp add 7 g of trisodium citrate, and for each 100 g of lemon pulp 20 g of trisodium citrate. Blend the mixture for 2 minutes. With a pH indicator paper, check the pH of the mixture, which should be between 4·5 and 5. Take for analysis an accurately weighed portion of the pulp mixture containing not more than 100 g of pulp (107 g of orange or grapefruit pulp mixture and 120 g of lemon pulp mixture correspond to 100 g of fruit pulp).

Extraction of thiabendazole—Place in the 500-ml stainless-steel container of the homogeniser the accurately weighed amount of chopped peel or pulp mixture. Add exactly 3 ml of

chloroform per gram of peel or pulp. Locate the shaft with the cutting blades in the container. Weigh the latter with its contents, note the total amount and mince for 15 minutes. Mince the peels at high speed to an impalpable purée and the pulps at moderate speed. Do not remove the cutting blades from the container. Adjust, by adding chloroform, the mass of the container with its contents to that previously noted so as to compensate for the loss of chloroform that may have occurred during mincing. Avoid any change in the volume of the extract during the subsequent operations. Transfer the chloroform extract through a fine sieve into a 500-ml separating funnel and compress slightly the residue of peel extract on the sieve in order to obtain the maximum amount of extract. Allow the phases to separate. Filter the chloroform phase through an adequate, folded filter-paper (Whatman No. 2) containing about 25 g of anhydrous sodium sulphate. Collect the filtrate, which should be clear, in a ground-glass stoppered graduated cylinder. Note the volume of the filtrate (generally about 85 per cent. of the volume of chloroform used for the extraction), each 3 ml of which represents 1 g of peel or pulp taken for analysis. Transfer the filtrate quantitatively into a 350-ml flat-bottomed flask with a short neck, connect it to the condenser via the Vigreux column and concentrate the extract at atmospheric pressure to a volume of about 10 to 15 ml.

Separation of thiabendazole from the bulk of extractive substances—Add to the extract 20 ml of water and exactly 5 ml of 0·1N hydrochloric acid, and boil to eliminate completely the remaining chloroform. Detach the flask from the concentration apparatus and boil the acidic mixture while exposed, concentrating it to approximately 10 ml. Cool and transfer the mixture quantitatively into a 25-ml calibrated flask, rinsing the flask with distilled water, and make the volume up to the mark with distilled water. Mix the solution and filter carefully through a folded filter-paper (Whatman No. 2). The filtrate is almost colourless and should be sufficiently clear to avoid the formation of an emulsion during subsequent extraction with chloroform.

Clean-up of interfering substances—Place exactly 20 ml of the filtrate, which contains 4 ml of 0·1N hydrochloric acid, into a 100-ml separating funnel. Wash the filtrate several times (usually four or five times) with 1-ml portions of chloroform, shaking the separating funnel gently to avoid formation of emulsions, until two consecutive washes remain colourless. Decant the washes carefully into a separating funnel, extract them with exactly 5 ml of 0·1N hydrochloric acid and discard them. Transfer the acidic phase quantitatively into the separating funnel containing the filtrate, rinse the empty funnel with a few drops of distilled water and add the rinsings to the combined acidic solutions. The solution obtained contains 9 ml of 0·1N hydrochloric acid and a certain amount of interfering substances that react with sodium hydroxide, some of which change on neutralisation from colourless to yellow. Make the solution alkaline to pH 10·5 to 11 by adding, from a microburette, 0·9 ml of 1N sodium hydroxide solution, continuing the addition carefully until the colour of the solution turns yellow. Check the pH of the mixture with pH indicator paper and adjust it to between 10·5 and 11, as described under Adjustment of pH. Then add 0·5 ml of 5 per cent. sodium hydrogen carbonate solution and check the pH with the pH indicator paper; the final pH should be about 9·5. Extract the mixture four times with 5-ml portions of chloroform and decant the chloroform phases carefully into a 100-ml separating funnel, discarding the aqueous phase.

Extract the combined chloroform phases with exactly 10, 10 and 5 ml of 0·1N hydrochloric acid. After the first two extractions, decant the chloroform phase into a clean separating funnel and discard it after the third extraction. Add quantitatively the second and the third acidic extracts to the first extract, rinsing the empty funnel with a few drops of water. Wash the combined acidic extracts with three 1-ml portions of chloroform, collect the washes in a separating funnel and extract them with 5 ml of 0·1N hydrochloric acid. Discard the chloroform phase and quantitatively transfer the acidic phase into the funnel containing the combined acidic extracts. With a microburette, add 3·0 ml of 1N sodium hydroxide solution and check the pH of the mixture, which should be between approximately 10·5 and 11. Then add 0·5 ml of 5 per cent. sodium hydrogen carbonate solution and check the pH of the mixture, which should be about 9·5. Extract it with five 4 to 5-ml portions of chloroform, collect the chloroform phases in a 25-ml calibrated flask and make the volume up to the mark. Finally, add anhydrous sodium sulphate, mix, allow the mixture to stand and determine the thiabendazole content of the chloroform extract.

DETERMINATION OF THE THIABENDAZOLE CONTENT OF THE EXTRACT—

A solution of thiabendazole in chloroform gives a characteristic ultraviolet absorption spectrum with a wide absorption band between 255 and 330 nm, with a maximum at 302 to 303 nm and two plateaux, one slight at 295 nm and the other distinct at 310 to 315 nm (Fig. 1).

Preparation of standard graphs—Prepare two standard graphs by using the 10 and 40-mm path length cells. Dissolve 20 mg of thiabendazole in a few millilitres of chloroform and make the volume up to 100 ml. From this solution, prepare, by suitably diluting with chloroform, several solutions containing increasing amounts of thiabendazole ranging from 0·1 to 13 μg ml^{-1} and record their ultraviolet spectra against chloroform in the reference cell, by using the 40-mm cells for solutions containing 0·1 to 3·2 μg ml^{-1} and the 10-mm cells for solutions containing 0·5 to 13 μg ml^{-1}. Determine the absorbances at 302 to 303 nm and prepare the standard graphs, each of which is a straight line. The absorptivity, $E^{1\%}_{1cm}$ value, for thiabendazole in chloroform solution was found to be 1080.

Determination of thiabendazole—Record the spectrum of the chloroform extract against chloroform in the reference cell, by using the 40-mm cell for extracts containing less than 1 to 2 μg ml^{-1} of thiabendazole and the 10-mm cell for more concentrated extracts. If the absorbance at 302 to 303 nm in the 10-mm cell exceeds 1·4, dilute the extract adequately and note the degree of dilution. Measure the absorbances at 302 to 303 nm and determine from the standard graph the concentration of thiabendazole in the extract, correcting for dilution if necessary. Calculate the content of the peel or pulp taken for the analysis as follows:

Thiabendazole content in peel or pulp $= \frac{a \times 25 \times 25 \times 3}{20 \times b}$ p.p.m., where a μg ml^{-1} is the concentration of this compound in the chloroform extract, and b ml is the volume of the chloroform filtrate taken. Calculate the thiabendazole content in the whole fruit if necessary.

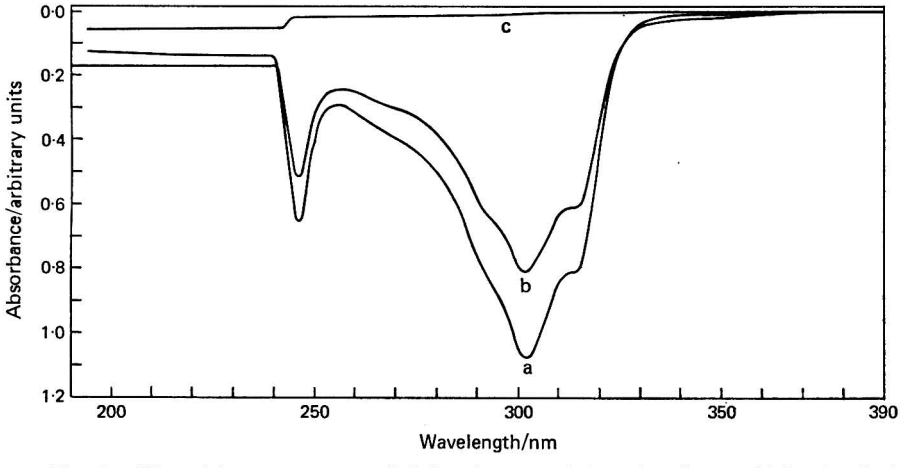

Fig. 1. Ultraviolet spectra recorded in 10-mm path length cells: a, thiabendazole in chloroform solution; b, extract from fruit treated with thiabendazole; and c, extract from untreated fruit

The ultraviolet spectrum for the treated fruits is shown in Fig. 1. If, accidentally, the extract still contains trace amounts of interfering substances, the absorbance at 330 nm may increase and exceed 0·1 and the slope of that portion of the spectrum between 330 and 390 nm increases. In this event purify the extract once more as follows: transfer into a 100-ml separating funnel an aliquot of the chloroform extract and proceed as described above for the combined chloroform phases, extracting the chloroform phase with exactly 10, 10 and 5 ml of 0·1N hydrochloric acid. Continue as described and determine the thiabendazole content of the chloroform extract.

DISCUSSION

SENSITIVITY—

The method permits the determination of small amounts of thiabendazole of the order

of 0·10 p.p.m. in 100 g of peel or pulp and of 0·03 p.p.m. in the whole fruit. The sensitivity can be increased by taking larger samples of peel or pulp.

ACCURACY—

Thiabendazole was determined in the peel and pulp (Table I) of untreated fruits to which known amounts of the compound had been added: 1 ml of a solution of thiabendazole in chloroform was dispersed on 100 g of chopped peels or pulps, placed in the container of the homogeniser, for 10 to 15 minutes before the addition of the required amount of the solvent to the peels and before the addition of the trisodium citrate to the pulps. The recovery of added thiabendazole varies between 94·1 and 103·0 per cent. (Table I). It was also determined in several samples originating from the same mixture of finely chopped peel of the treated fruit (Table II). The results show that the deviations are slight.

TABLE I

RECOVERY OF THIABENDAZOLE ADDED TO PEELS AND PULP OF NON-TREATED FRUITS

Thiabendazole added to 100 g of peel or pulp/ μg	Recovery, per cent.		
	Shamouti oranges	Grapefruit	Lemons
Peels—			
0	0	0	0
20	96·0	103·0	95·6
40	100·6	98·2	102·0
100	101·1	98·5	—
	101·5	100·2	—
250	94·4	96·0	98·0
	99·2	99·0	99·2
1250	96·0	97·3	95·6
	97·0	99·9	100·8
2500	94·1	—	96·0
	99·2	—	98·3
Pulp—			
0	0	0	0
20	102·0	97·0	94·8
	98·5	95·8	96·7
50	94·1	97·5	96·5
	99·6	98·3	100·1

VALIDITY OF THE METHOD—

Solvent—The solubility of thiabendazole at 25 °C in various solvents was determined. For this purpose a saturated solution in a given solvent was filtered, the ultraviolet spectrum

TABLE II

DETERMINATION OF THIABENDAZOLE IN PEELS OF TREATED FRUITS

100-g samples taken from the same mixture of finely chopped peel

Mixture	Sample No.	Thiabendazole found, μg	Deviation from average value, per cent.
A	1	179	−0·72
	2	180	−0·16
	3	182	+0·94
	Average	180·3	
B	1	271	−3·4
	2	285	+1·5
	3	286	+1·9
	Average	280·7	
C	1	920	−2·2
	2	939	−0·24
	3	965	+2·5
	Average	941·3	

TABLE III

SOLUBILITY OF THIABENDAZOLE IN VARIOUS SOLVENTS AT 25 °C

Solvent	Solubility of thiabendazole/mg ml^{-1}
Chloroform	3·2
Ethyl acetate	1·8
Hexane	0·001
Cyclohexane	0·001
Hydrochloric acid/N	
0·1	20·5
0·05	7·9
0·02	3·2
0·01	3·0
0·001	1·0
0·0001	0·05
Sodium hydroxide solution/N	
1	2·0
0·1	0·25
0·05	0·12
0·01	0·05
Citric acid solution, per cent.	
5·0	6·3
2·5	3·6
1·0	2·8

of the filtrate, adequately diluted if necessary, was recorded against this solvent in the reference cell and the maximum absorbance of the characteristic peak noted. The concentration of this compound in the filtrate was calculated according to the absorbance of a known amount dissolved in the respective solvent. It was found that thiabendazole was more soluble in chloroform than in ethyl acetate (Table III).

It has been reported[3,4] that during the extraction of thiabendazole with ethyl acetate the fruit pulp or whole-fruit blend forms emulsions with the solvent, which have to be broken by centrifugation or some product added in order to reduce[3] or prevent their formation. It is to be noted that no emulsion formation was observed with the use of the Vir-Tis "45" homogeniser for mincing the pulp or the whole fruit with chloroform.

Extraction of thiabendazole from citrus peels and pulps—Citrus fruits contain considerable amounts of organic acids (chiefly citric acid) and their salts, which are mainly localised in fruit juices. The total acidity, expressed in grams of citric acid per 100 ml of citrus juice, corresponding to free and combined organic acids, is about 1 to 2 g for orange and grapefruit juice and 5 to 7 g for lemon juice. Citrus fruit peels contain a comparatively small amount of organic acids. The pH of orange and grapefruit juice is about 3, of lemon juice about 2, and of citrus peel about 5. In order to establish the conditions for the quantitative extraction of thiabendazole from citrus fruit, the solubility of this compound in aqueous solutions of citric acid was determined as described above (Table III), and its distribution behaviour between chloroform and various aqueous solutions of pure citric acid, and of mixtures of citric acid and trisodium citrate, was studied (Table IV). The distribution behaviour was expressed as the fraction of total solute which distributes itself in the non-polar phase for equal volumes of solvent.[8] The fraction found in the chloroform phase was calculated as a percentage of the total amount of thiabendazole.

The fraction found in the chloroform phase depends upon the pH of the aqueous solution (Table IV). When the pH is between 4·5 and 5, about 98 per cent. of the thiabendazole partitions into the chloroform, thus enabling almost all of it to be extracted by equilibrating one volume of the aqueous phase with three volumes of chloroform, as described in the procedure. At least 3·06 g of trisodium citrate dihydrate per 1 g of citric acid (2 mol of trisodium citrate per 1 mol of citric acid) should be added in order to increase the pH of the solution to between 4·5 and 5, which justifies the addition of trisodium citrate to the pulp of the fruit. Owing to the relatively high pH of citrus peel, the addition of trisodium citrate to the peel is unnecessary. The presence of an excess of trisodium citrate does not affect the distribution behaviour of thiabendazole.

Separation of thiabendazole from the bulk of other extractives—This compound is soluble in hydrochloric acid solutions (Table III). Complete elimination of chloroform from the acidic mixture is necessary in order to precipitate the extractive substances, which are insoluble in hydrochloric acid. The acidic mixture should be heated to dissolve the thiabendazole, which is eventually incorporated in the insoluble bulk of other extractives, and concentrating the mixture contributes to the elimination of a large proportion of the steam-volatile extractives.

TABLE IV

DISTRIBUTIONS OF THIABENDAZOLE BETWEEN CHLOROFORM AND VARIOUS AQUEOUS SOLUTIONS FOR EQUAL VOLUMES OF SOLVENTS

200 μg dissolved in 20 ml of chloroform and solution shaken for 10 minutes with 20 ml of aqueous solution; pH of aqueous solutions determined by pH meter before partition with chloroform

Aqueous solution	pH		Thiabendazole, per cent. of total, found in chloroform phase
Citric acid, per cent. *m/V*			
5	1·8		11
2	2·05		19
1	2·2		26
0·5	2·4		36
0·1	2·8		60
0·01	3·4		86
0·005	3·7		94
0·002	4·0		98
0·001	4·4		99
Citric acid, 1 per cent. *m/V* (*a*) + trisodium citrate dihydrate/mg per 100 ml (*b*)		Molar ratio *b/a*	
0	2·2	0:1	26
191	2·8	0·12:1	53
765	3·8	0·5:1	89
1531	4·4	1·0:1	97
3062	4·5	2·0:1	99–100
3828	5·1	2·5:1	99–100
Hydrochloric acid	1·0		3
	2·0		19
	3·0		65
	4·0		93
	5·0		98–99
	6·6		98–99
Sodium hydroxide	9·4		98–99
	1·07		98
	11·8		95
	12·7		84
	14·0		48

Clean-up of the remaining interfering substances—In order to establish the conditions that permit the separation of thiabendazole from the remaining interfering substances, its solubility in hydrochloric acid and sodium hydroxide solutions was determined (Table III), and its distribution behaviour between chloroform and the various aqueous solutions was studied (Table IV). The results show that under the conditions of the procedure described, it is possible to extract it almost quantitatively from the chloroform phase with 0·1N hydrochloric acid, and from the aqueous phase, at pH 5 to 10, with chloroform. It was found that in order to eliminate most of the interfering substances, the aqueous phase must be made slightly alkaline, to a pH of 10·5 to 11. The subsequent addition of the sodium hydrogen carbonate buffer solution decreases the pH to about 9·5. The ultraviolet spectrum for untreated fruit (Fig. 1) shows that the interfering substances are practically eliminated.

The low recoveries of thiabendazole[3,4] added to the fruit may have been caused in part by the extraction and clean-up procedures used by these authors. Its partition behaviour between various aqueous solutions on the one hand and ethyl acetate or chloroform on the other was found to be similar for the two solvents.

Biphenyl and 2-phenylphenol, currently used for post-harvest treatment of citrus fruit, did not interfere in the determination, as these products are eliminated during the various stages of the procedure. On the other hand, methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate (benomyl), an experimental fungicide that may replace thiabendazole, and its degradation product methyl 2-benzimidazole carbamate, interfere in the determination by the method described. However, it is unlikely that thiabendazole and benomyl would be used simultaneously for citrus fruit treatment.

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References

1. Eckert, J. W., *Wld Rev. Pest Control*, **1969**, **8**, 116.
2. "Pesticide Analytical Manual," Section 2, Food and Drug Administration, Washington, D.C., **1969**, pp. 120 to 242.
3. Mestres, R., Campo, M., and Tourte, J., *Trav. Soc. Pharm. Montpellier*, **1970**, **30**, 193.
4. Norman, S. M., Fouse, D. C., and Craft, C. C., *J. Ass. Off. Analyt. Chem.*, **1972**, **55**, 1239.
5. Kroller, E., *Dt. LebensmittRdsch.*, **1971**, **67**, 229.
6. Hey, H., *Z. Lebensmitteluntersu.-Forsch.*, **1972**, **149**, 79.
7. Gunther, F. A., *Residue Rev.*, **1969**, **28**, 83.
8. Beroza, M., Inscoe, M. N., and Bowman, M. C., *Ibid.*, **1969**, **30**, 1.

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Photometric Determination of Manganese in Water by Using *o*-Tolidine

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Oxidation products of manganese react with *o*-tolidine to produce a yellow colour, which can be utilised for the photometric determination of manganese. Prior to the determination, divalent manganese is converted into the higher valency forms by oxidation with atmospheric oxygen in alkaline solution in the presence of magnesium and iron(III) salts.

In addition to neutron-activation analysis, which together with amperometric titration is considered to be the most sensitive method for the determination of manganese,[1] the most frequently used processes for its determination in water remain the photometric methods. These methods include the measurement of the violet coloration of permanganate ions,[2] following oxidation of manganese to its higher valency form; less frequently used are the coloured products obtained by reaction of manganese(II) ions with some organic reagents, *e.g.*, formaldoxime,[3] pyridine-2,6-dialdoxime and pyridine-2,6-diacetoxime,[4] 1,2-(pyridylazo)-2-naphthol,[5] thenoyltrifluoracetone[6] and 8-mercaptoquinoline.[7] The reactions of manganese(II) ions, however, lack the desired selectivity while the permanganate colour lacks the necessary sensitivity in the determination of very low concentrations of the element.

The required selectivity and adequate sensitivity can be easily attained by converting manganese into a given higher valency form that is detectable by some redox indicators. For this purpose *o*-tolidine,* which has a yellow oxidised form, and 3,3′-dimethylnaphthidine, the oxidised form of which is red, are applied. *o*-Dianisidine was discounted because it gave an insufficiently sensitive reaction. Although the use of indicators for the determination of the oxidised forms of manganese present in waters is not difficult,[8] the oxidation of manganese(II) ions to a suitable form that would react with the indicator in such a way as to prevent an undesirable co-reaction with the oxidising agent used posed a problem.

Of the oxidising substances that could be used, which react in homogeneous solution, *e.g.*, persulphate,[9] iodate[10] and bromate,[11] or in heterogeneous medium, *e.g.*, lead dioxide,[12] silver(II) oxide[13] and sodium bismuthate,[14] none is suitable because even when they are removed, either in their original form (PbO_2, $NaBiO_3$, etc.), or in the form of one of their insoluble salts, such as $Hg(IO_4)_2$,[15] the sensitive redox indicator still reacts with the residual concentrations of the oxidising agents, to an extent depending on their solubility.

Although ammonium persulphate can be essentially removed by decomposition when the mixture is boiled, the stability of the ions of the higher oxidation forms of manganese depends on a residual persulphate concentration in the final measured solution. The only suitable alternative to these methods was the oxidation of manganese by atmospheric oxygen in alkaline medium to a higher valency form that could be determined by photometry with *o*-tolidine and 3,3′-dimethylnaphthidine, reagents with which molecular oxygen does not react under the given conditions.

Experimental

Conditions for the oxidation of manganese by atmospheric oxygen in alkaline medium and the reactions of its higher valency forms with *o*-tolidine or 3,3′-dimethylnaphthidine in acidic solution were investigated by measuring the absorbance of the corresponding colours on a Pulfrich photometer with ELPHO-2 supplementary equipment in 2-cm cells. For the yellow colour from *o*-tolidine an S-44 filter (440 nm) was used, while for the red colour from

* *o*-Tolidine is a carcinogen—see cautionary note on p. 134.

3,3′-dimethylnaphthidine an S-53 filter (530 nm) was used. A colloidal suspension of hydrated manganese dioxide, solutions with a known manganese(II) ion content and solutions containing a mixture of known concentrations of manganese(II), calcium(II), magnesium(II) and iron(III) ions were used as standard solutions.

The concentrations of manganese given for the calibration graphs are related to the measured solutions.

The colloidal $2{\cdot}5 \times 10^{-5}$ M solution of hydrated manganese dioxide was freshly prepared by mixing 10·0 ml of 0·001 M potassium permanganate solution with 20·0 ml of 0·003 M manganese sulphate solution, then neutralising the mixture with 7·2 ml of 0·1 N sodium hydroxide solution and adding distilled water to 1 litre. In the procedure, the volume of the colloidal hydrated manganese dioxide solution used was adjusted to 25 ml, it was then acidified with 5 ml of 3 N perchloric acid and, after adding 1 ml of 0·05 per cent. 3,3′-dimethylnaphthidine solution or 2·5 ml of 0·2 per cent. *o*-tolidine solution, made up to 50 ml. The intensity of the resulting colour was measured immediately.

The corresponding calibration graphs are shown in Fig. 1 (A) and Fig. 3 (A). The stability of polyvalent manganese ions in acidic medium, demonstrated on solutions to which the reagent was added at different time intervals after acidification of the mixture, is proved in Fig. 2. The effect of various ways of treating the colloidal hydrated manganese dioxide solution is indicated in Table I, which also shows the development of colour intensity with time.

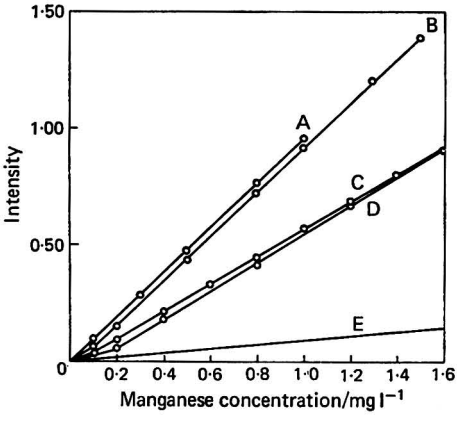

Fig. 1. Colour intensity of the oxidation product of 3,3′-dimethylnaphthidine with higher valency Mn compounds in acidic medium and colour intensity of $KMnO_4$. Cell 2 cm; filter S-53 (530 nm). A, Colloidal $MnO_2.xH_2O$. In resultant solution: $HClO_4$ (0·3 N) and 0·001 per cent. of 3,3′-dimethylnaphthidine. B, Mn^{III} + Mn^{IV} hydroxides prepared by oxidation of Mn^{II} with atmospheric oxygen in ammoniacal medium. In resultant solution: H_3PO_4 (0·7 M) and 0·001 per cent. of 3,3′-dimethylnaphthidine. C, Mn^{III} + Mn^{IV} hydroxides prepared by oxidation of Mn^{II} with atmospheric oxygen in ammoniacal medium in the presence of magnesium, calcium and iron(III) ions, the reagent being added after acidification with dilute phosphoric acid. In resultant solution: H_3PO_4 (0·7 M) and 0·001 per cent. of 3,3′-dimethylnaphthidine. D, As for C, the reagent being added in dilute phosphoric acid solution. E, $KMnO_4$ solution

With the solutions of known manganese(II) ion content, the problem was to reconcile the reaction conditions for converting the manganese(II) ions, by oxidation in an ammoniacal medium with molecular oxygen, into a mixture of insoluble hydrated manganese(III) and (IV)

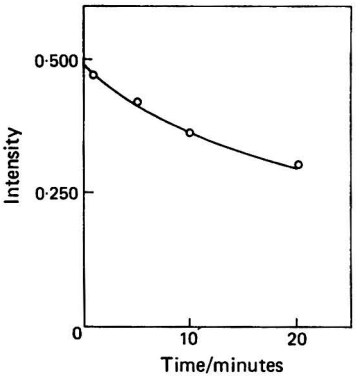

Fig. 2. Reduction of Mn^{IV} ions. Cell 2 cm; filter S-53 (530 nm). In resultant solution: 0·5 mg l^{-1} of manganese as $MnO_2.xH_2O$, $HClO_4$ (0·3 M) and 0·001 per cent. of 3,3′-dimethylnaphthidine

oxides, with suitable chemical and physical properties, which, under simple conditions of determination, are easily reproducible. Following the dissolution of the hydroxide precipitate in phosphoric acid the released manganese(III) and manganese(IV) ions oxidise the reagent, producing a colour the intensity of which is measured photometrically.

In the investigation, 25 ml of the selected manganese(II) salt solution were taken, to which were added 4 ml of dilute ammonia solution (1 + 1) and the mixture was immersed in a water-bath for 10 minutes. After reaching room temperature, the sample was acidified with 10 ml of dilute phosphoric acid (1 + 3) and the colour developed by adding 1 ml of 0·05 per cent. 3,3′-dimethylnaphthidine solution in acetic acid. In the reaction with *o*-tolidine, 10 ml of reagent dissolved in phosphoric acid solution were added [0·5 g of *o*-tolidine in 1 litre of dilute phosphoric acid (1 + 3)]. After making the solution up to 50 ml with distilled water, the colour intensity was noted.

The calibration graphs for this procedure are shown in Fig. 1 (B) and Fig. 3 (B).

TABLE I

DEVELOPMENT WITH TIME OF COLOUR PRODUCED BY REACTION OF *o*-TOLIDINE WITH MANGANESE(IV) IONS IN ACIDIC SOLUTION

Contents of measured solution: 0·3 N $HClO_4$, 0·01 per cent. *o*-tolidine, 0.75×10^{-5} M $MnO_2.xH_2O$

Time after addition of *o*-tolidine/minutes	Absorbance			
	1*	2*	3*	4*
1	0·598	0·602	0·410	0·627
2	0·610	0·633	0·440	0·637
3	0·612	0·650	0·463	0·635
4	0·610	0·655	0·485	0·635
5	0·610	0·655	0·502	0·635
7	0·610	0·645	0·528	0·635
10	0·608	0·640	0·555	0·622
15	0·598	0·630	0·582	0·615
20	0·585	0·630	0·588	0·612

* Treatment of colloidal $MnO_2.xH_2O$ solution before reaction: 1, freshly prepared solution without further treatment; 2, solution boiled for 1 minute; 3, solution boiled for 10 minutes; and 4, solution after standing for 24 hours.

For the solution of a mixture of ions [the solution containing amounts of calcium chloride, magnesium chloride and ammonium iron(III) sulphate in addition to a manganese(II) salt], the procedure is identical with that for manganese(II) alone. The corresponding results are given in Tables II and III.

In accordance with the results obtained, the individual concentrations of the calcium(II), magnesium(II) and iron(III) ions were adjusted so as to ascertain whether possible variations in the content of these ions within the wide concentration ranges indicated would have any marked effect on the oxidation of manganese, on the reaction of its higher oxidation forms with the reagent used and thus on the intensity of the final colour produced. That there is no marked effect is proved for 3,3′-dimethylnaphthidine with the optimum content of 5 mg of iron(III), 20 mg of calcium(II) and 20 mg of magnesium(II), Fig. 1 (C), and for *o*-tolidine containing 5 mg of iron(III) and 20 mg of magnesium(II), Fig. 3 (C). Table IV shows the stability of the colour produced for one concentration of manganese.

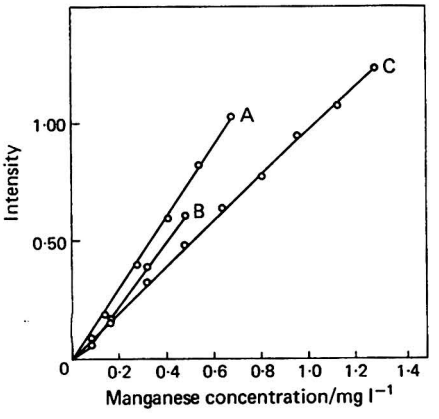

Fig. 3. Colour intensity of the oxidation product of *o*-tolidine with higher valency manganese compounds in acidic medium. Cell 2 cm; filter S-44 (440 nm). A, Colloidal $MnO_2.xH_2O$. In resultant solution: $HClO_4$ (0·75 M) and 0·01 per cent. of *o*-tolidine. B, $Mn^{III} + Mn^{IV}$ hydroxides prepared by oxidation of Mn^{II} with atmospheric oxygen in ammoniacal medium. In resultant solution: H_3PO_4 (0·7 M) and 0·01 per cent. of *o*-tolidine. C, $Mn^{III} + Mn^{IV}$ hydroxides prepared by oxidation of Mn^{II} with atmospheric oxygen in ammoniacal medium in the presence of magnesium, calcium and iron(III) ions. In resultant solution: H_3PO_4 (0·7 M) and 0·01 per cent. of *o*-tolidine

Results and discussion

The dependence of absorbance on the concentration of manganese is linear up to 800 μg per litre of measured solution, *i.e.*, up to 16000 μg per litre of the analysed sample. The probable error of determination is ± 12 μg per litre of sample.

The method described was tested by analysing a series of samples of surface and waste-waters. Manganese was also determined on the same samples photometrically as permanganate following concentration and oxidation with persulphate[2] (Table V).

Of the possible methods for converting manganese(II) into a higher valency form, the only one that is suitable, bearing in mind the subsequent photometric determination with *o*-tolidine or 3,3′-dimethylnaphthidine, is the oxidation of manganese(II) ions with atmospheric oxygen in alkaline medium, as the oxygen in molecular form does not oxidise these reagents. In addition, this oxidation method can be used for this purpose because, when using alkaline solutions, it produces suspensions of manganese compounds of stable higher valency, whereas mineral acid solutions of manganese(III) and manganese(IV) ions are unstable, the oxidation equivalent decreasing with time (Fig. 2). The preparation of the manganese(III) and (IV) compounds should therefore be carried out in neutral or alkaline media, from which the active component is released in ionic form by a mineral acid added immediately before the colour reagent.

TABLE II

EFFECT OF IRON(III), MAGNESIUM AND CALCIUM IONS ON COLOUR INTENSITY PRODUCED BY REACTION OF MANGANESE(III) AND (IV) IONS, EVOLVED FROM MANGANESE(III) AND (IV) HYDROXIDES, WITH 3,3′-DIMETHYLNAPHTHIDINE IN ACIDIC SOLUTION

Manganese concentration in measured solution 0·80 mg l^{-1}. The concentrations of interfering ions refer to the content in 50 ml of the measured solution

Interfering ion/mg	Absorbance
Mg(II)—	
0·000	0·790
0·001	0·765
0·005	0·685
0·010	0·650
0·020	0·600
0·040	0·385
0·060	0·318
0·100	0·290
0·200	0·288
0·300	0·283
0·500	0·270
Fe(III)—	
0·000	0·790
0·01	0·765
0·05	0·711
0·10	0·650
0·20	0·612
0·50	0·560
1·00	0·542
2·00	0·528
5·00	0·505
Ca(II)—	
0·00	0·790
1·00	0·785
2·00	0·776

Fe(III) 5 *mg*—

Ca(II)/mg .. / Mg(II)/mg	Absorbance 0	10	20	30	50
0	0·495	0·640	0·645	0·680	0·720
10	0·407	0·490	0·505	0·510	0·518
20	0·395	0·435	0·465	0·467	0·487
30	0·381	0·433	0·470	0·485	0·503
40	0·395	0·462	0·482	0·490	0·502

Mg(II) 20 *mg* + *Ca(II)* 20 *mg*—

Fe(III)/mg	Absorbance
2·5	0·490
5·0	0·470
10·0	0·488
15·0	0·520

From the manganese(II) solutions, after having rendered them alkaline, amorphous manganese(II) hydroxide is separated, which is quickly oxidised by the oxygen present in the solution to hydrated manganese(III) and (IV) oxides of complicated composition, arranged hexagonally in a stratified lattice that is penetrated by more or less irregular layers of manganese(II) hydroxide. With an increasing ratio of oxygen to manganese, this system of hydrated manganese(III) and (IV) oxides changes to the stable δ-form of hydrated $MnO_2.xH_2O$.[16] If only micro-amounts of manganese(II) hydroxide are present in the solution,

TABLE III

EFFECT OF IRON(III), MAGNESIUM AND CALCIUM IONS ON COLOUR INTENSITY PRODUCED BY REACTION OF MANGANESE(III) AND (IV) IONS, EVOLVED FROM MANGANESE(III) AND (IV) HYDROXIDES, WITH *o*-TOLIDINE IN ACIDIC SOLUTION

Manganese concentration in measured solution 0·40 mg l^{-1}. The concentrations of interfering ions refer to the content in 50 ml of the measured solution

Fe(III) 5 *mg*—

Ca(II)/mg .. / Mg(II)/mg	Absorbance 0	10	20	30	50
0	0·410	0·470	0·475	0·485	0·485
10	0·385	0·393	0·392	0·392	0·392
20	0·397	0·397	0·396	0·393	0·392
30	0·398	0·397	0·395	0·402	0·400
40	0·408	0·415	0·413	0·420	0·425

Mg(II) 20 *mg* + *Ca(II)* 20 *mg*—

Fe(III)/mg	Absorbance
2·5	0·395
5·0	0·395
10·0	0·397
15·0	0·400

then the excess of oxygen oxidises them almost immediately to $MnO_2.xH_2O$, as would powerful oxidising agents such as hydrogen peroxide or bromine.

In order to obtain reproducible results, the conditions must be capable of ensuring that the precipitate of manganese hydroxides produced during the determination always has the same chemical composition and physical structure, which affect the rate of dissolution in acid. This rate, together with the instability of the manganese(III) and manganese(IV) ions in acidic medium, is a significant factor in achieving useful results. The change with time of the colour intensity resulting from the reaction of manganese(III) and (IV) oxides of the same concentrations, but having different physical structures, with *o*-tolidine, shown in Table I, proves the effect of this factor and at the same time casts doubt on the possibility of using the reagent for the determination of higher valency forms of manganese in natural waters in accordance with the method proposed earlier.[8] The physical state of these manganese compounds is not known and hence they cannot even be roughly reproduced by using synthetic solutions. The calibration graph is thus plotted under conditions that may not correspond to the state of the sample.

After testing various procedures, among which aeration of the basic reaction solution of manganese(II) salts at room temperature did not prove satisfactory, the reaction mixture had to be heated in a water-bath, after adding ammonia or an alkali-metal hydroxide, in order to obtain manganese(III) and (IV) compounds with reproducible physical properties. In order to obtain a quantitative oxidation of manganese, the presence of oxygen in strongly alkaline medium is evidently not sufficient, but it is necessary in order to achieve the thermal destruction of the strongly hydrated shells of the manganese(II) ions.[16] As a result of prolonged boiling, as well as ageing of the precipitates of hydroxides and basic salts of manganese, an additional destruction of their micelles occurs, causing a change in their resistance to attack by acids. For this reason, the use of alkali-metal hydroxides is less suitable because the precipitates of hydroxides and hydrated oxides of manganese formed with them become only slightly soluble in acids after being heated.

TABLE IV

STABILITY WITH TIME OF COLOUR PREPARED BY REACTION OF MANGANESE(III) AND (IV) IONS WITH *o*-TOLIDINE

Manganese concentration in measured solution 0·40 mg l^{-1}

Time after addition of *o*-tolidine/minutes	Absorbance
1	0·435
2	0·433
3	0·431
4	0·429
5	0·427
7	0·423
10	0·412
15	0·397

The determination of manganese by the proposed method is dependent on the presence of iron(III) and magnesium ions, which reduce the absorbance of the resultant colour (Tables II and III). Within a certain concentration range, however, this influence is constant so that the interference of these cations can be eliminated by the addition of salts to each sample so as to bring the concentration within this range. When the above elements are present in various combinations the conditions become much more complicated. For example, in contradiction to the above, the addition of an iron(III) salt in the presence of magnesium ions increases the absorbance of the resultant colour. Calcium ions do not interfere to such an extent as manganese(II) or iron(III) ions, although they do decrease the solubility of the precipitate of manganese hydroxides in acidic solution.

In order to mask iron(III) ions, it is advisable to use phosphoric acid for acidifying the sample, because the iron(III) hydroxide precipitate separated from a hot ammoniacal solution dissolves readily in this acid. If, however, the precipitation is carried out in an alkali-metal hydroxide solution, the rate of dissolution of the separated manganese hydroxides and iron(III) hydroxide decreases considerably. In the presence of an adequate concentration of magnesium ions, a precipitate of unknown composition is produced, which contains

both iron and magnesium. This white precipitate is not only readily soluble in phosphoric acid but also in an excess of alkaline EDTA solution, thus differing from iron(III) hydroxide, which does not dissolve in EDTA solution. This observation disproves an earlier theory,[17] which stated that the appearance of the white precipitate obtained on addition of ammonia solution to the iron(III) salt in the presence of a large amount of magnesium ions is explicable by a subsequent coprecipitation on particles of separated iron(III) hydroxide.

TABLE V

COMPARISON OF METHODS FOR THE DETERMINATION OF MANGANESE IN SURFACE WATER AND SEWAGE INVOLVING *o*-TOLIDINE AND PERMANGANATE PROCEDURES

Sample number	Manganese by *o*-tolidine method/mg l^{-1}	Manganese by permanganate method/mg l^{-1}	Δ (mg l^{-1} of manganese)
1	0·179	0·168	+0·011
2	0·218	0·234	−0·016
3	0·080	0·087	−0·007
4	0·343	0·325	+0·018
5	0·110	0·112	−0·002
6	0·039	0·044	−0·005
7	0·124	0·136	−0·012
8	0·231	0·211	+0·010
9	0·101	0·094	+0·007
10	0·051	0·062	+0·011

Substantial changes in the solubility of hydroxides of the higher valency forms of manganese, precipitated in the presence of iron(III) and magnesium salts in acidic solution, compared with the solubility of the same hydroxides when these salts had not been present or had been added later, indicate that in the example under consideration the influence of some linkages of extraneous ions in the lattice of manganese hydroxides cannot be excluded.

By adding iron(III) and magnesium salts to the manganese(II) salt solution, the solubility of the corresponding hydroxides separated in hot phosphoric acid increases, so that, in order to render the solution alkaline, sodium hydroxide can also be used. By choosing a suitable concentration of magnesium(II) and iron(III) ions, it is possible to achieve also a degree of independence of the resulting colour intensity with *o*-tolidine and 3,3′-dimethylnaphthidine for the given ions in a fairly wide concentration range.

o-Tolidine has a number of advantages compared with 3,3′-dimethylnaphthidine,[18] so that it is included in the final method for manganese determination. The determination is more sensitive and the calibration graph is completely linear from zero manganese concentration. The greater solubility of *o*-tolidine in dilute phosphoric acid permits the addition of this solution in one operation, whereas, when using 3,3′-dimethylnaphthidine, the precipitate of hydroxides has to be dissolved first in strong acid before the addition of the reagent solution in acetic acid.

METHOD

REAGENTS—

o-Tolidine solution—*o*-Tolidine (0·5 g) is dissolved in 1 litre of dilute phosphoric acid (1 + 3).

CAUTION—*o*-Tolidine is a carcinogen and appropriate precautions should be taken in its use [see S.I. 1967 No. 879, "Factories—The Carcinogenic Substances Regulations 1967," H.M. Stationery Office (reprinted 1970), and "Precautions for Laboratory Workers who Handle Carcinogenic Aromatic Amines," The Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, 1966 (reprinted 1971)].

Dilute ammonia solution (1 + 1).

Ammonium iron(III) sulphate solution—The solid (43·17 g) is dissolved in distilled water and the solution acidified with 5 ml of concentrated sulphuric acid; this acidic solution is then filtered and made up to 1 litre with distilled water. One millilitre of this solution contains 5 mg of iron(III).

Magnesium chloride solution—$MgCl_2.6H_2O$ (83·60 g) is dissolved in distilled water and the solution made up to 1 litre.

Standard manganese solution—A 0·2748-g amount of anhydrous manganese sulphate (prepared from the hydrated salt ignited at 500 °C) is dissolved in 10 ml of hot dilute sulphuric acid (1 + 4) and the solution made up to 1 litre with distilled water; 1 ml of the solution contains 0·100 mg of manganese(II). The working solutions are prepared by dilution of this standard solution.

APPARATUS—

Pulfrich photometer with ELPHO-2.
S-44 filter (440 nm).
Measuring cells, 2 cm.

PROCEDURE—

To 25·0 ml of the test solution containing up to 40 μg of manganese(II) in a 50-ml calibrated flask, 1·0 ml of ammonium iron(III) sulphate solution and 2·0 ml of magnesium chloride solution are added. The addition of these salts can be carried out simultaneously by use of a combined solution. The solution is then made alkaline by addition of 4·0 ml of dilute ammonia solution (1 + 1), thoroughly mixed and immersed in a water-bath for 10 minutes. After cooling the solution to room temperature in a stream of tap water, 10·0 ml of *o*-tolidine solution in dilute phosphoric acid are added. The volume is then made up to 50 ml with distilled water, the solution mixed and, within 3 minutes, the colour intensity read on the photometer.

The determination requires the initial solution to be approximately neutral. If no manganese in bivalent form is present in the original sample, although present bound at higher valencies in hydrated oxides, the sample has to be evaporated to dryness first with 5 ml of aqua regia, which also destroys the organic matter, thus causing turbidity in the sample. The evaporation with aqua regia can be repeated if necessary. The residue is then evaporated twice with about 3 ml of concentrated hydrochloric acid so as to expel the oxides of nitrogen and finally it is moistened with a few drops of dilute hydrochloric acid and dissolved in water while still hot. The calibration graph is plotted under the same working conditions.

INTERFERENCES—

When using the proposed method, no interference is observed in the presence of the following ions: 400 mg of magnesium(II), 100 mg of iron(III) (not including the amounts present in the salts added during the procedure), 400 mg of calcium(II), 400 mg of ammonium, 400 mg of sodium, 400 mg of potassium, 300 mg of carbonate, 1400 mg of chloride, 2000 mg of nitrate, 4000 mg of sulphate and 200 mg of phosphate in 1 litre of sample. Iron(II) does not interfere up to a concentration of 40 mg l^{-1}; at higher iron(II) ion concentrations the resultant colour fades as the dissolved oxygen becomes exhausted in the reaction between iron(II) hydroxide and oxygen, being then deficient for the oxidation of manganese(II).

CONCLUSION

For the determination of manganese at concentrations up to 1·6 mg l^{-1}, a rapid, sensitive and precise photometric method using *o*-tolidine has been presented. The method is based on the oxidation of manganese(II) ions in alkaline solution by atmospheric oxygen to manganese(III) and (IV) hydroxides of suitable chemical and physical properties, which are reproducible under the conditions of the determination. By dissolving these hydroxides in phosphoric acid, manganese(III) and (IV) ions are evolved and react with the reagent to give a corresponding colour, which is evaluated photometrically. The relationship between the concentration and the colour produced is linear. If no divalent manganese is present in the solution under test, the cation is converted into this form from manganese(III) and (IV) compounds prior to the determination by evaporation with aqua regia and hydrochloric acid. The determination of manganese with *o*-tolidine is superior to an alternative method that involves the use of 3,3′-dimethylnaphthidine.

This paper is dedicated to the seventieth birthday of Dr. Arnošt Okáč, Professor of Analytical Chemistry at the University of Brno and a Member of the Czechoslovak Academy of Sciences.

References

1. Meinke, W. W., *Science, N.Y.*, 1955, **121**, 177.
2. "Standard Methods for the Examination of Water and Waste-water," Twelfth Edition, American Public Health Association, New York, 1965.
3. Gottlieb, A., and Hecht, F., *Mikrochemie*, 1950, **35**, 337.
4. Hartkamp, H., *Angew. Chem.*, 1960, **72**, 349.
5. Donaldson, E. M., and Inman, W. R., *Talanta*, 1966, **13**, 489.
6. De, A. K., and Rahaman, S., *Analyt. Chem.*, 1963, **35**, 159.
7. Bankovskij, J. A., Ievinch, A. F., and Lukcha, E. A., *Zh. Analit. Khim.*, 1959, **14**, 222.
8. Morgan, J. J., and Stumm, W., *J. Amer. Wat. Wks Ass.*, 1965, **57**, 107.
9. Nydahl, F., *Analytica Chim. Acta*, 1949, **3**, 144.
10. Waterbury, G. R., Hayes, A. M., and Martin, D. S., *J. Amer. Chem. Soc.*, 1952, **74**, 15.
11. Purdy, W. C., and Hume, D. W., *Analyt. Chem.*, 1955, **27**, 256.
12. Watters, J. J., and Kolthoff, J. M., *J. Amer. Chem. Soc.*, 1948, **70**, 2455.
13. Lingane, J. J., and Davis, D. G., *Analytica Chim. Acta*, 1956, **15**, 201.
14. Přibil, R., and Hornychová, A., *Chemické Listy*, 1950, **44**, 101.
15. Smith, G. F., McHard, J. A., and Olsen, K. L., *Ind. Engng Chem., Analyt. Edn*, 1936, **8**, 350.
16. Ettel, V., and Vepřek-Šiška, J., *Chemické. Listy*, 1963, **57**, 785.
17. Patton, J., and Reeder, W., *Analyt. Chem.*, 1956, **153**, 1026.
18. Malý, J., and Fadrus, H., *Hydrochemia, Bratislava*, 1970, **7**, 3.

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Determination of Organic Acids of Low Relative Molecular Mass (C_1 to C_4) in Dilute Aqueous Solution

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A method for determining organic acids of low relative molecular mass present in low concentrations (5×10^{-5} M) in polluted waters is presented. The method is based on the conversion of the acids into their benzyl esters via lipophilic tetra-n-butylammonium salts. The benzyl esters are detected and determined by gas - liquid chromatography.

A RECENT report[1] concerning the identification of organic acids of low relative molecular mass prompted us to present a study that we have carried out on the determination of organic acids by gas chromatography of their benzyl ester derivatives.

The increasing interest in substances that contribute to the biochemical oxygen demand of polluted waters has focused attention on methods for determining volatile organic acids of low relative molecular mass. Those of principal interest to the forest products industry are formic, acetic, propionic, butyric, glycollic and lactic acids, which are formed during the processing of wood. Our aim was to determine the above acids in one procedure by a simple and accurate method.

The determination of free fatty acids in aqueous solution by gas - liquid chromatography has been reported.[2–5] This method has a high sensitivity,[5] but also a number of drawbacks, the most serious being the difficulty in determining all the acids of interest in one chromatogram. Other complications depend on the fact that water is used as solvent, which may give rise to well known complications such as the appearance of ghost peaks that interfere in the quantitative evaluation of the chromatogram. Therefore, methods involving the formation of derivatives were sought. Concentration of solutions would appear to be the first step in the assay procedure, but the fairly high volatility of all of the acids except glycollic acid precludes evaporation of water as a technique to this end. A common technique is to neutralise the sample and isolate the acids as their alkali-metal salts, after removal of the water by evaporation or freeze-drying. The salts are usually converted into their corresponding methyl esters or trimethylsilyl esters, compounds that are extremely volatile and therefore difficult to determine by gas chromatography.[6–8] It would be convenient to use less volatile esters of the same acids, for example, the benzyl ester of formic acid, which has a boiling-point of 203 °C. The quantitative preparation of such esters presents a number of problems. The familiar reaction of carboxylic acids with benzyl bromide in a solution of potassium carbonate in acetone is slow and there seems to be no established quantitative method for the determination of C_1 to C_4 carboxylic acids that is sufficiently accurate, simple and rapid. However, C_2 to C_{10} straight-chain carboxylic acids in the form of *p*-bromophenacyl and *p*-phenylphenacyl esters have been quantitatively determined in dilute aqueous solution.[9]

Organic anions can be extracted from an aqueous phase into a lipophilic phase by means of positively charged counter ions, such as the tetra-n-butylammonium ion. If the lipophilic phase contains a reactive alkyl halide, such as benzyl bromide, the anion acts as a nucleophile and participates in a displacement reaction.[10] A similar reaction has recently been used in the determination of phenols and higher fatty acids in aqueous solutions.[11] The extraction constants for most hydrophilic anions, such as those of formic, acetic, propionic, glycollic and lactic acids, are small and the reactions are therefore slow.

If the procedure is modified so that the aqueous sample solution is neutralised with tetra-n-butylammonium hydroxide and then evaporated, the acids remain in the residue as their tetra-n-butylammonium salts, which are very soluble in aprotic solvents; in acetone containing benzyl bromide, a nucleophilic substitution reaction starts almost immediately, and the formation of benzyl esters is quantitative. The reaction mixture can be analysed by gas chromatography without further preparation. This procedure is the basis of the method described in this paper.

METHOD

APPARATUS—

A gas chromatograph with a flame-ionisation detector and a device for temperature programming, for example, a Perkin-Elmer, Model 900, and an electronic integrator, for example, a Hewlett-Packard, Model 3770A, were used.

A 2-m × 2-mm stainless-steel column containing 3 per cent. butane-1,4-diol succinate polyester on acid-washed Chromosorb W, mesh size 120 to 140, was used, and the chromatographic conditions (single column operation) were as follows: injector temperature, 160 °C; detector temperature, 190 °C; nitrogen carrier gas flow-rate, 30 ml min^{-1}; initial column temperature, 120 °C; period at initial temperature, 17 minutes; programme rate, 2·5 °C min^{-1}; and final temperature, 150 °C.

A pH meter equipped with a combined electrode, for example, a Radiometer PHM 63 with a GD 2301 C electrode, and a 10 × 100-mm glass column containing Dowex 50W-X8 ion-exchange resin, 100 to 200 mesh, in the H^+ form, were also used.

REAGENTS—

Tetra-n-butylammonium hydroxide solution, 40 per cent.—Prepare aqueous solutions at concentrations of 0·7 and 0·03 M, and standardise them with standard hydrochloric acid, using the pH meter to detect the end-point.

Benzyl bromide—Merck AG, Darmstadt.

CAUTION—Benzyl bromide is a powerful lachrymator and should be stored and used in a fume chamber. Benzyl bromide can be destroyed by pouring it into dilute ammonia solution.

n-Hexanoic acid, (internal standard) solution, 0·1 M—Purify the commercially available acid by distillation. Mix 11·62 g (0·10 mol) of the purified acid with 250 ml of water. Adjust the pH of the mixture to 8·9 with the 0·7 M tetra-n-butylammonium hydroxide solution, using the pH meter to detect the end-point. Transfer the reaction mixture to a 1000-ml calibrated flask and make the volume up to the mark with distilled water.

In order to standardise the solution, transfer 10·0 ml to a titration flask and add 25·0 ml of 0·1 M standard hydrochloric acid. Titrate the excess of hydrochloric acid with 0·1 M standard sodium hydroxide solution.

Acetone—Analytical-reagent grade.

Carboxylic acids—Analytical-reagent grade acids were generally used for the analyses.

CALIBRATION—

It is necessary to determine the "response factor" for each of the benzyl esters. In order to ensure the highest precision of the results, the values of these factors should be checked frequently; they are determined as follows. Prepare 0·1 M solutions of all the relevant acids by diluting 0·1 mol of each acid to 1 litre with distilled water in a calibrated flask. Standardise 10 ml of the 0·1 M solutions with carbon dioxide free 0·1 M standard sodium hydroxide solution. Transfer V_x µl of each standardised C_x M solution of acid (see Table II) into a 25-ml beaker and add V_1 µl of the internal standard solution. Dilute the mixture to about 15 ml with distilled water so as to obtain enough liquid for the glass electrode. Carry out the analysis exactly as under Procedure except for the ion-exchange step. After injection into the gas chromatograph, read from the integrator the relative peak area for the ester in the sample (A_x) and for the internal standard (A_1). Calculate the molar response factor of the ester from the equation

$$f_x = \frac{V_x C_x A_1}{V_1 C_1 A_x} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where f_x is the molar response factor of the ester; V_1 µl the volume of the internal standard solution; and C_1 mol l^{-1} the concentration of the internal standard solution.

PROCEDURE—

Transfer 10 ml of the aqueous sample solution to the ion-exchange column in order to remove the metal cations. Wash the column three times with 5 ml of water and add the washings to the sample. With the use of the pH meter as an end-point detector, titrate the

combined eluates to pH 8 with the standard tetra-n-butylammonium hydroxide solution and record the volume of titrant consumed (V_t ml). Calculate the volume of internal standard solution (V_1 µl) required for the sample from the equation

$$V_1 = k \cdot \frac{C_t \times V_t}{C_1} \times 10^3 \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where k is an arbitrary factor, to be chosen within the range $0{\cdot}1 < k < 1$ so as to obtain a reasonable standard peak area; C_t mol l^{-1}, the concentration of the tetra-n-butylammonium hydroxide solution; and C_1 mol l^{-1}, the concentration of the n-hexanoic acid internal standard solution.

Add the calculated amount of internal standard solution to the neutralised solution and transfer the combined solutions into a 25-ml pear-shaped flask. Concentrate the solution to a syrup in a rotating evaporator and dissolve the residue in acetone; the volume (V_a ml) required is obtained from the equation

$$V_a = \frac{C_t V_t}{C_r} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where C_r is the optimum concentration of acids in the reaction mixture. This concentration is normally about 10 mmol l^{-1}, in which case

$$V_a = 100\, C_t V_t \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

With a 10-µl syringe, add V_b µl of benzyl bromide (*i.e.*, in slight excess) over the equivalent of the sum of tetra-n-butylammonium hydroxide and the internal standard. Calculate V_b from the equation

$$V_b = \frac{171{\cdot}04\,(C_t V_t \times 10^3 + V_1 C_1)}{1{\cdot}438 \times 10^3} + \Delta V_b \quad .. \quad .. \quad .. \quad (5)$$

Equations (2) and (5) give

$$V_b = \frac{171{\cdot}04\, C_t \times V_t\,(1 + k)}{1{\cdot}438} + \Delta V_b \quad .. \quad .. \quad .. \quad (6)$$

where ΔV_b is the slight excess of benzyl bromide. In practice, this equation reduces to

$$V_b = 120\, C_t V_t\,(1 + k) \quad .. \quad .. \quad .. \quad .. \quad (7)$$

Allow the reaction mixture to stand for 2 hours in order to ensure that the reaction reaches completion. Inject 1 µl of the reaction mixture, read the peak areas from the integrator and calculate the concentration of each acid in the aqueous sample from the general equation

$$C_x = \frac{f_x \times V_1 \times C_1 \times A_x \times M_x}{V \times A_1} \quad .. \quad .. \quad .. \quad .. \quad (8)$$

where C_x mg l^{-1} is the concentration of the acid in the aqueous sample; M_x, the relative molecular mass of the acid; and V ml, the volume of the aqueous sample to be analysed.

Discussion and results

In several trials with different gas-chromatographic stationary phases, including OV-1, OV-17 and ECNSS-M, a 3 per cent. butane-1,4-diol succinate polyester column gave the best resolution of the benzyl esters. Fig. 1 shows a typical chromatogram with n-hexanoic acid as the internal standard.

In order to confirm that the yield of the esters is quantitative, the peak area of the benzyl ester formed from a known amount of acetic acid and the peak area for an equivalent amount of pure benzyl acetate were compared with those of equivalent amounts of the internal standard. The ratios of the peak areas of both acetates to that of the internal standard were identical in the two cases.

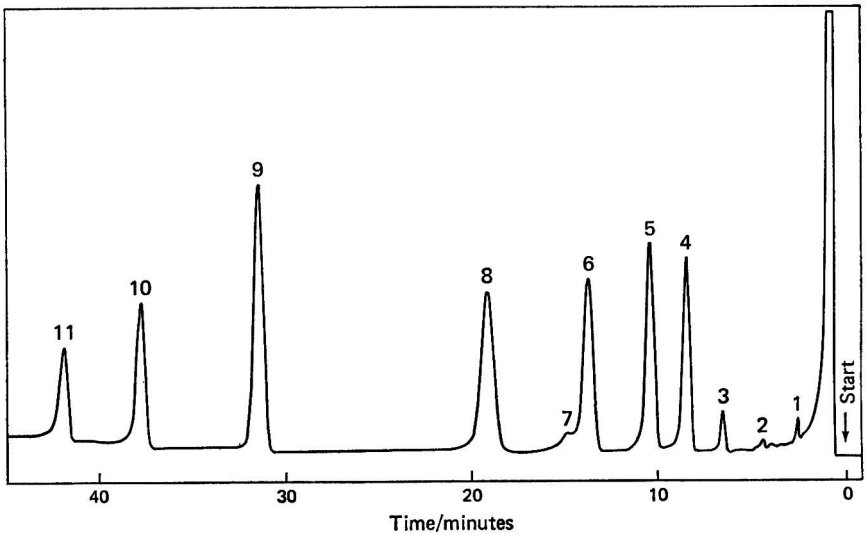

Fig. 1. Gas chromatogram of benzyl compounds in acetone (10 μmol ml^{-1}) from tetra-n-butylammonium salts. The acids are at 10^{-3} M concentration in the original aqueous solution. 1 and 2, contaminants. Benzyl compounds: 3, bromide; 4, formate; 5, acetate; 6, propionate; 7, alcohol; 8, butyrate; 9, n-hexanoate (internal standard); 10, lactate; and 11, glycollate. Chromatographic conditions as previously described: sample size, 1 μl; detector attenuation, × 200; and chart speed, 10 mm min^{-1}

The precision of the molar response factors calculated from five chromatograms obtained with the same solution is shown in Table I, the concentrations of the esters in the injection solution being approximately 25 μmol ml^{-1}. The standard deviation of the response factors is satisfactory except for the glycollate, with which the peak is broader and the tails are longer.

The values of the molar response factors given in Table II are the means of five injections of each sample with concentrations ranging from 10 to 25 μmol ml^{-1}. The coefficients of variation are slightly higher than those shown in Table I. The average response factors differ slightly from those given in Table I because two different columns and detectors were used; the values in this table were obtained on a 2 per cent. butane-1,4-diol succinate polyester column, the initial and final temperatures of which were comparatively low. The precision is somewhat lower for the propionate than for the other components (except for the glycollate), because of slight interference from benzyl alcohol.

TABLE I

RELATIVE MOLAR RESPONSE FACTORS (BENZYL n-HEXANOATE = 1) FOR VARIOUS ESTERS, DETERMINED ON ONE ESTER MIXTURE

Chromatographic conditions—

Column	2 per cent. butane-1,4-diol succinate polyester	Programme rate	2·5 °C min^{-1}
		Final temperature ..	170 °C
Initial column temperature ..	100 °C	Injector temperature ..	160 °C
Period at initial temperature	14 minutes	Detector temperature ..	190 °C

	Formate	Acetate	Propionate	Butyrate	Lactate	Glycollate
Injection 1	1·78	1·42	1·43	1·17	1·59	3·20
2	1·80	1·45	1·46	1·19	1·60	3·32
3	1·78	1·43	1·43	1·16	1·56	2·79
4	1·76	1·42	1·42	1·17	1·59	2·88
5	1·84	1·47	1·47	1·21	1·58	2·91
Mean	1·79	1·44	1·44	1·18	1·59	3·02
Maximum	1·84	1·47	1·47	1·21	1·60	3·32
Minimum	1·76	1·42	1·42	1·16	1·58	2·79
Standard deviation ..	0·030	0·022	0·022	0·020	0·015	0·228
Coefficient of variation, per cent.	1·6	1·4	1·4	1·6	0·9	7·2

TABLE II

RELATIVE MOLAR RESPONSE FACTORS (BENZYL n-HEXANOATE = 1) DETERMINED ON DIFFERENT ESTER MIXTURES

Chromatographic conditions—

Initial column temperature ..	100 °C	Final temperature ..	180 °C
Period at initial temperature ..	17 minutes	Injector temperature ..	160 °C
Programme rate	2·5 °C min^{-1}	Detector temperature ..	190 °C

Amount of each ester/μmol—	Acetone/ ml	Concentration/ μmol ml^{-1}	Formate	Acetate	Propionate	Butyrate	Lactate	Glycollate
5	0·5	10	1·99	1·43	1·56	1·30	1·48	2·76
10	0·5	20	1·91	1·49	1·49	1·24	1·53	3·24
50	2	25	1·96	1·43	1·45	1·26	1·56	2·79
100	4	25	1·95	1·50	1·53	1·27	1·57	2·91
150	6	25	1·91	1·43	1·44	1·20	1·53	2·81
200	8	25	1·96	1·48	1·52	1·25	1·58	3·14
Mean			1·95	1·46	1·50	1·25	1·54	2·94
Maximum			1·99	1·50	1·56	1·30	1·58	3·24
Minimum			1·91	1·43	1·44	1·20	1·48	2·76
Standard deviation			0·031	0·034	0·047	0·033	0·037	0·197
Coefficient of variation, per cent.			1·5	2·2	3·0	2·5	2·2	6·5

The molar response factors are calculated for concentrations of each acid with the limits 10 to 25 μmol per millilitre of acetone, which is equivalent to 10 to 25 μmol per 10 ml of each acid in water, *i.e.*, 10^{-3} to $2{\cdot}5 \times 10^{-3}$ M. However, Fig. 2 shows a chromatogram obtained with an effluent sample from a plant producing fibre building board. The injection solution contained approximately 1 μmol per 0·5 ml of acetone, which indicates a concentration of 10^{-4} M in the original effluent.

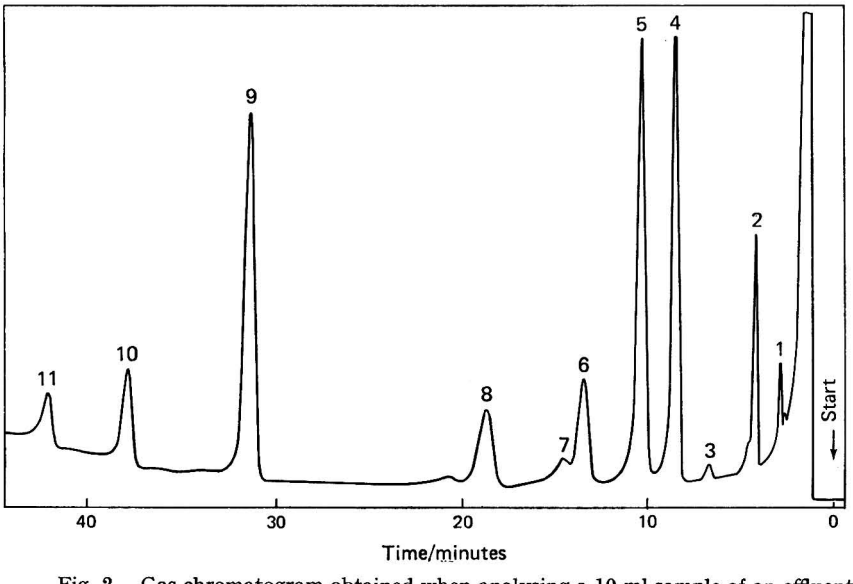

Fig. 2. Gas chromatogram obtained when analysing a 10-ml sample of an effluent from a plant producing fibre building board. Peak compounds: 1 and 2, contaminants. Benzyl compounds (with concentration of compound in acetone, μmol ml^{-1}; and acid concentration in original aqueous solution, mol l^{-1}): 3, bromide (—, —); 4, formate (3·4, $1{\cdot}7 \times 10^{-4}$); 5, acetate, (2·8, $1{\cdot}4 \times 10^{-4}$); 6, propionate (0·8, 4×10^{-5}); 7, alcohol (—, —); 8, butyrate (0·8, 4×10^{-5}); 9, n-hexanoate (internal standard) ($2{\cdot}0, 2{\cdot}0 \times 10^{-4}$); 10, lactate (0·8, 4×10^{-5}); and 11, glycollate ($1{\cdot}0, 5 \times 10^{-5}$). Chromatographic conditions as previously described: sample size, 1 μl; detector attenuation, × 64; and chart speed, 10 mm min^{-1}

The lowest concentrations of acid determined in the original sample by the procedure were of the order of 5×10^{-5} M. In such cases, the volume of original sample taken was 10 ml and the volume of acetone added 0·5 ml. If higher sensitivity is required, the sample volume can be increased or the acetone volume decreased. However, if the sample contains large amounts of acids other than those required to be determined, the residue to be dissolved will be fairly large and the volume of acetone has to be increased. In the absence of such interfering acids the sample volume can be increased without difficulty and the sensitivity could be increased to about 5×10^{-6} M.

The presence of benzyl alcohol probably results from small amounts of carbonates and carbon dioxide present in the original sample. After cation exchange and neutralisation, the carbonic acid is converted into a stable tetra-n-butylammonium salt, which reacts with benzyl bromide to form an unstable monofunctional benzyl hydrogen carbonate; this compound in turn forms benzyl alcohol and carbon dioxide. When aqueous solutions with increasing concentrations of sodium hydrogen carbonate were analysed as described, the benzyl alcohol peaks increased in proportion, thus supporting the suggested explanation.

When an acidic aqueous solution with a total acid concentration below 0·05 M was neutralised to pH 8·0 with the tetra-n-butylammonium hydroxide solution, all of the acids were converted into the anionic form and, after evaporating the solution, the residual product consisted of lipophilic salts, because of the highly lipophilic nature of the counter ion. It is important to check the pH carefully with a meter in order to avoid degradation by alkali of the esters formed later in the procedure. The tetra-n-butylammonium salts are soluble in most organic solvents and the anions act as excellent nucleophiles, which participate in displacement reactions whereby benzyl esters are formed:

$$R_1COOH + (R_2)_4N^+OH^- \rightleftharpoons R_1COO^-(R_2)_4N^+ + H_2O$$

$$R_1COO^- + C_6H_5CH_2Br \rightarrow R_1COOCH_2C_6H_5 + Br^-$$

A slight excess of benzyl bromide was used so as to ensure that the reaction reached completion; the benzyl bromide peak in the chromatogram did not interfere with the ester peaks. The reaction mixture was stable, but after a few days the benzyl formate started to decompose slowly, as indicated by an increase in the benzyl alcohol peak and a corresponding decrease in the benzyl formate peak.

The various esters in a reaction mixture were determined by mass spectrometry with a Perkin-Elmer 270 instrument; the mass spectra showed typical ester fragmentation. The spectra of all the acid esters had a base peak at *m/e* 91 due to the tropylium ion, and they all showed clearly the molecular ions.

The authors are indebted to Mrs. Maivor Karlsson for her skilful assistance.

References

1. Aronvic, S. M., Crowell, E. P., Whalen, D. M., and Burnett, B. B., *TAPPI*, 1971, **54**, 1963.
2. Ustinovskaya, I. A., Gavrilina, L. Ya., and Malakhow, V. V., *Zav. Lab.*, 1968, **35**, 553.
3. Ottenstein, D. M., and Bartley, D. A., *Analyt. Chem.*, 1971, **43**, 952.
4. Mahadevan, V., and Stenroos, L., *Ibid.*, 1967, **39**, 1652.
5. van Huysteen, J. J., *Wat. Res.*, 1970, **4**, 645.
6. Merriman, M. M., Choulett, H., and Brink, D. L., *TAPPI*, 1966, **49**, 34.
7. Monzie-Guillement, D., Rooux, H., and Monzie, P., *Tech. Rech. Papet.*, 1966, **7**, 37.
8. Martin, G. E., and Swinehart, J. S., *J. Gas Chromat.*, 1968, **6**, 533.
9. Umeh, E. O., *J. Chromat.*, 1971, **56**, 29.
10. Brändström, A., *Svensk Kem. Tidskr.*, 1970, **82**, 5 and 32.
11. Ehrsson, H., *Acta Pharm. Suecia*, 1971, **8**, 113.

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Book Reviews

VIBRATIONAL SPECTROSCOPY OF SOLIDS. By P. M. A. SHERWOOD. *Cambridge Monographs in Physical Chemistry*, 1. Pp. xii + 254. Cambridge: Cambridge University Press. 1972. Price £5·90.

The advent of laser Raman spectroscopy and its utility for the examination of solids, together with the increasing availability of far-infrared spectrometers, has yielded about a decade of work on the vibrational spectra of solid samples. Despite this effort, no book exists which meets the needs of the chemist faced with the understanding and interpretation of solid-state spectra. Hitherto the theory has been the realm of the solid-state physicist and his language is not too readily understandable to the average practising chemical spectroscopist. This book sets out to provide such an interpretation and in this task the author has succeeded admirably.

A detailed account is given of a simple model lattice and the various types of lattice vibrations. The treatment is extremely lucid and greatly clarified by the inclusion of numerous diagrams. It will find considerable use by all levels of research workers in vibrational spectroscopy and is strongly recommended to them. Although partly addressed to senior undergraduates in chemistry, it will be useful only to those few who undertake special courses on the topic.

In addition to the main theme, the two final chapters discuss second-order vibrational effects and spectroscopic effects other than vibrational transitions. While the chemist will find it of value to have some brief discussions of excitons, plasmons and magnons (Chapter 6), some of the more important topics, such as Brillouin scattering, receive barely a page. A page each devoted to hydrogen bonding and to the pressure dependence of vibrational modes is also of little value—these should either have been expanded or omitted altogether. The section on crystal disorder and defects including matrix studies could also profitably have been greatly expanded, even to the extent of a separate chapter.

However, this criticism should not detract from the value of this book and in particular its timeliness. It fills a growing gap in vibrational spectroscopy and it should be well received by solid-state chemists and spectroscopists.

H. E. HALLAM

RADIOISOTOPE LABORATORY TECHNIQUES. Third Edition. By R. A. FAIRES and B. H. PARKS. Pp. xvi + 312. London: Butterworths. 1973. Price £3·20.

A new edition of this well known work has appeared after an interval of thirteen years. It is a useful introductory guide to the subject by two experienced practical teachers, and can be recommended to all workers entering the field as well as to libraries in secondary schools, polytechnics and universities.

The text has been partly revised to incorporate SI units, many recent books have been added to the supplementary reading lists and an extra chapter on liquid scintillation counting (by R. D. Stubbs) has been inserted. These changes have not always been consistent. For example, Appendix 1, which lists physical constants and definitions, does not make clear which units are SI units and which are not. The Curie is not an SI unit and should ultimately be replaced by disintegrations/s or disintegrations s^{-1}. The non-SI radiation unit known as the Rad can easily be confused with the abbreviation rad (for radian) in the SI system. My students, brought up to use the SI system, are confused by the use of terms "range" and "half-thickness" for absorbers when these quantities are measured in g cm^{-2}. Other small inconsistencies that may puzzle students include the confusion of the neutrino and the antineutrino (pp. 7 and 20), the distinction between resistance and resistivity (p. 105) and the meaning of "flow of air" measured in m/min (pp. 61 and 65). Several books published prior to 1960 still remain in the book lists although they have been superseded by later works, but I was sorry to see that the scholarly works of De Soete, Gijbels and Hoste (on activation analysis) and of Tölgyessy, Braun and Kyrs (on isotope dilution) have not been mentioned.

The presentation is carried out with admirable simplicity and is far more readable than the average scientific book. There are a few places where recent material has been imperfectly merged into the original text. For example, the section on detection devices (Chapters 10 and 11) fails to mention the excitation of electrons in semiconductors as an important property of ionising radiation, except in a few paragraphs at the end of Chapter 11. This compares with the 20 pages devoted to proportional counters and Geiger counters, which are now little used for quantitative work. I failed to notice any reference to the relatively poor resolution of well-type scintillation

counters compared with undrilled crystals. Another omission concerns practical methods of preventing the frequent breakdowns of electronic equipment in tropical conditions, especially at high humidities. A section on this subject would be particularly useful for readers in developing countries, where earlier editions have had a wide circulation.

Butterworths have re-set the type and made new blocks for the figures; I noticed only three misprints. The paper is of inferior quality to that of the second edition, and it is a sign of the times that the price of this third edition is more than double that of the second. H. J. M. BOWEN

GAS AND LIQUID CHROMATOGRAPHY ABSTRACTS 1972. Edited by C. E. H. KNAPMAN and R. J. MAGGS. Indexed by D. E. HILLMAN and N. J. EARL. Sponsored by THE GAS CHROMATOGRAPHY DISCUSSION GROUP OF THE INSTITUTE OF PETROLEUM. Pp. xviii + 251. Barking, Essex: Applied Science Publishers Ltd., on behalf of The Institute of Petroleum. 1973. Price £8.

These abstracts, which have been published continually since 1958, continue to be the most useful source of information to practising chromatographers. One hundred and fifty two journals from all over the world have been abstracted for this issue.

The excellent subject index ensures that information can be retrieved rapidly from the abstracts. Seven major subject divisions, each divided into further subdivisions, achieve the maximum number of cross-references. Sections are devoted to the following self-explanatory subjects: Theory; Definitions and retention data; Apparatus and technique; Carrier gas and column packing material; Sample type; Applications and specialised separations; and finally a section on Related methods and techniques. An author index is also provided to the 853 references cited.

This volume includes a separate section on liquid chromatography, in which 300 abstracts are cited.

"Gas Chromatography Abstracts" is particularly valuable and the volunteer abstractors should be highly praised for the efficiency of their work; many of the abstracts in this volume are from journals first published in 1972. In future the abstracts will be published in a quarterly journal, which will make them available to libraries at the same time as they are available to members of the Gas Chromatography Discussion Group of the Institute of Petroleum. P. B. STOCKWELL

N-NITROSO COMPOUNDS, ANALYSIS AND FORMATION. PROCEEDINGS OF A WORKING CONFERENCE HELD AT THE DEUTSCHES KREBSFORSCHUNGSZENTRUM, HEIDELBERG, FEDERAL REPUBLIC OF GERMANY, 13–15 OCTOBER 1971. Edited by P. BOGOVSKI, R. PREUSSMANN and E. A. WALKER. Technical Editor for IARC: W. DAVIS. *IARC Scientific Publication No.* 3. Pp. xvi + 140. Lyon: International Agency for Research on Cancer. 1972.

These proceedings contain contributions from most of the major research groups involved in the topic of the analysis and formation of *N*-nitroso compounds. The Summaries of Discussion and the Reports of the Sub-Committees give a clear over-all picture of the state of the art at that time.

Unfortunately, much of the impact of this book has been lost by the long interval between the time of the Symposium and the appearance of these Proceedings. As so often happens in a topic where there is a high level of research effort, much of the "new" work reported here has since been published in full elsewhere and many of the views expressed have probably been modified in the light of later experience.

It is to be hoped that the Proceedings of the meeting held in Lyon in October, 1973, will appear within a more acceptable period of time. G. M. TELLING

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A Highly Selective Direct Colorimetric Procedure for the Determination of Zirconium in Steel with Arsenazo III by Using a Pressure-digestion Technique for Sample Dissolution

Zirconium in steel is determined colorimetrically with arsenazo III in 7·5 M perchloric acid solution. Chemically inert forms of zirconium, which remain unchanged after conventional treatment with acid, are dissolved by means of a pressure-digestion technique. Results obtained with six British Chemical Standard steel samples show excellent precision and accuracy. Interference studies with fifteen metals commonly found in steel indicate that the procedure is applicable to a wide range of steels. Concentrations of zirconium in excess of 0·001 per cent. *m/m* in steel samples can be determined.

A. ASHTON, A. G. FOGG and D. THORBURN BURNS

Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire, LE11 3TU.

Analyst, 1974, **99**, 108–113.

Determination of an Isomeric Impurity in Samples of Morantel Tartrate by Gas - Liquid Chromatographic Analysis of the Products of a Controlled Degradation Procedure

A procedure is described for the determination of the tartrate salt of the 4-methylthienyl isomer of morantel, which is present in samples of morantel tartrate. The procedure involves a controlled degradative oxidation of the ethylene bond that converts morantel and the 4-methylthienyl isomer of morantel into 3-methylthiophene-2-carbaldehyde and 4-methylthiophene-2-carbaldehyde, respectively. Extraction of the two aldehydes is followed by the determination of their relative concentrations by a gas - liquid chromatographic procedure. It is shown that the ratio of the aldehydes given by gas - liquid chromatography is not significantly different from the ratio of morantel to the 4-methylthienyl isomer of morantel present in prepared mixtures of the two isomers. The method is shown to have good precision and to be applicable to morantel samples containing 0·10 to 7·00 per cent. *m/m* of the 4-methylthienyl impurity.

E. ADDISON, E. DAVISON and P. F. WADSWORTH

Pfizer Central Research, Pfizer Ltd., Sandwich, Kent.

Analyst, 1974, **99**, 114–119.

Determination of Thiabendazole in Citrus Fruits by Ultraviolet Spectrophotometry

A method for the ultraviolet spectrophotometric determination of thiabendazole in citrus fruit is described. It is extracted from the fruits by equilibrating the mashed peels or pulps with chloroform, the extract is acidified and the mixture concentrated in order to eliminate the chloroform and filtered. After making the filtrate slightly alkaline the thiabendazole is re-extracted with chloroform and determined by measuring the absorption of the extract at 302 to 303 nm. The procedure enables the satisfactory elimination of interfering substances to be achieved, and small amounts of thiabendazole of the order of 0·1 p.p.m. in the peel and the pulps and 0·03 p.p.m. in the whole fruit to be determined. The recovery of thiabendazole added to the peel or pulp of fruit varies between 94·1 and 103·0 per cent.

ANNA RAJZMAN

Division of Fruit and Vegetable Storage, Institute for Technology and Storage of Agricultural Products, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan, Israel.

Analyst, 1974, **99**, 120–127.

Photometric Determination of Manganese in Water by Using o-Tolidine

Oxidation products of manganese react with *o*-tolidine to produce a yellow colour, which can be utilised for the photometric determination of manganese. Prior to the determination, bivalent manganese is converted into the higher valency forms by oxidation with atmospheric oxygen in alkaline solution in the presence of magnesium and iron(III) salts.

JOSEF MALÝ and HUBERT FADRUS

Water Management Board, Vodohospodářská Správa, Brno, Czechoslovakia.

Analyst, 1974, **99**, 128–136.

Determination of Organic Acids of Low Relative Molecular Mass (C_1 to C_4) in Dilute Aqueous Solution

A method for determining organic acids of low relative molecular mass present in low concentrations (5×10^{-5} M) in polluted waters is presented. The method is based on the conversion of the acids into their benzyl esters via lipophilic tetra-n-butylammonium salts. The benzyl esters are detected and determined by gas - liquid chromatography.

PER OLOF BETHGE and KRISTER LINDSTRÖM

Swedish Forest Products Research Laboratory, Box 5604, S–114 86 Stockholm, Sweden.

Analyst, 1974, **99**, 137–142.

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