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Historical Survey of the Uses of Organic Compounds as Reagents in Analytical Chemistry

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Finally, the classic chelate-forming reagents for metals such as copper, cobalt, iron and nickel, are viewed in their historical context as contributing to the development of selective and sensitive analytical reagents.

Keywords: *Organic analytical reagents*; *history*

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Analyst, 1977, **102**, 793–803.

Application of the Inverse Zeeman Effect to Background Correction in Electrothermal Atomic-absorption Analysis

An electromagnet was placed around a specially designed graphite furnace atomiser and the Zeeman effect generated in the atomised sample was used to correct for background absorption generated by the matrix. The optical polarisation properties inherent in the Zeeman effect were utilised to generate two signals, one corresponding to the absorption of the atomic line plus background and the other to the background alone. A simple theoretical treatment was developed that related the observed atomic absorbance to a parameter dependent on magnetic flux density. This parameter, designated R in this work, is a function of the atomic line profile and the Zeeman splitting pattern of the line. The purpose-built apparatus incorporated facilities for simultaneous background correction by both the Zeeman and conventional deuterium-arc techniques.

Measurements were made of the dependence of R on magnetic flux density for silver, gold, cadmium, chromium, mercury, potassium, magnesium, manganese, sodium, nickel and lead in the range 2–9 kG. A reduction in the analytical sensitivity of up to 35% arising from the Zeeman correction procedure was found in some instances. Calibration graphs for lead and nickel were linear with concentration change over two orders of magnitude but were found to invert at high concentrations (about 300 $\mu g\ ml^{-1}$). Using the Zeeman correction system, a background absorbance of 2.0 was compensated to better than 0.005, while that achieved by a well adjusted deuterium arc system was approximately 0.02.

Keywords: *Atomic-absorption spectrophotometry*; *electrothermal atomisation*; *background correction*; *Zeeman effect*

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Analyst, 1977, **102**, 804–818.

Determination of Metallic and "Oxide" Copper in Ores

The selective determination of metallic copper in the presence of copper sulphides and oxides is performed by removal of these oxides or sulphides by means of a cyanide - hydrazine leach solution (pH 12 ± 0.2), washing the residue and dissolving the metallic copper in nitric acid. The copper in this solution is then determined by an atomic-absorption spectrophotometric or spectrophotometric (cuprizone) procedure. The recovery of metallic copper is about 98.6% and the average standard deviation ±0.003% for the range 0.011–0.142%.

The "oxide" copper is dissolved out of another portion of sample by means of an ammonia - ammonium chloride - sodium pyrophosphate leach. The solution is filtered and the copper(I) in the filtrate is oxidised with hydrogen peroxide and the absorbance of the filtrate is measured at 610 nm. The method determines "oxide" copper(I) and copper(II) and neither metallic nor trivalent iron interferes. The average standard deviation is ±0.003% for the range 0.01–1.15% of "oxide" copper.

Keywords: *Copper determination*; *ores*; *atomic-absorption spectrophotometry*; *spectrophotometry*

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Analyst, 1977, **102**, 819–824.

Ion-exchange Separation and Spectrophotometric Determination of Trace Amounts of Niobium in Silicate Rocks

A spectrophotometric determination of trace amounts of niobium in rocks and minerals is described. Elemental interferences emanating from the silicate rock matrix are discussed. An ion-exchange separation method is proposed in an attempt to avoid these interferences. A method involving sodium peroxide fusion and hydrochloric acid - hydrogen peroxide leaching is also described. Results for the determination of trace amounts of niobium in international standards of rocks and minerals are shown.

Keywords: *Niobium determination*; *silicate rocks*; *ion-exchange separation*; *spectrophotometry*

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Analyst, 1977, **102**, 825–830.

Spectrophotometric Method for the Determination of Boron in Glasses, Glazes and Ceramic Colours

A rapid method for the determination of boron in glasses, glazes and ceramic colours is described, which is based on spectrophotometric measurement of the boron - carminic acid complex, and interference effects by other elements present have been investigated. The method has been designed to give maximum accuracy for a spectrophotometric procedure, bearing in mind that up to 20% of boron oxide may be present and that the reagent is very sensitive.

Keywords: *Boron determination*; *glasses*; *glazes*; *ceramics*; *spectrophotometry*

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Analyst, 1977, **102**, 831–836.

Haematoxylin with Quaternary Ammonium Salts as Spectrophotometric Reagents for Tin(IV)

Haematoxylin with cetyltrimethylammonium bromide (CTAB) is proposed for the spectrophotometric determination of tin(IV). The addition of CTAB improved the colour reaction of tin(IV) with haematoxylin, resulting in better sensitivity and a longer linear range of the calibration graph. Similar results were obtained with cetyldimethylbenzylammonium chloride and cetylpyridinium bromide. Oxidised haematoxylin is not a suitable reagent for tin(IV) because of its poor stability in solution.

Keywords: *Tin(IV) determination*; *spectrophotometric reagents*; *haematoxylin*; *cetyltrimethylammonium bromide*

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Analyst, 1977, **102**, 837–841.

Method for the Separation of Antimony(III) from Antimony(V) Using Polyurethane Foam

Antimony(III) and antimony(V) in solution can be separated by adjusting the pH to 9.5 and shaking with sodium diethyldithiocarbamate (NaDDC) and polyurethane foam. Alternatively, the solution can be passed through a column of foam that has previously been treated with 5% *m*/*V* of NaDDC in carbon tetrachloride. The $Sb(DDC)_3$ complex retained by the foam can be eluted with acetone. DDC complexes of iron(III), mercury and silver are also absorbed, but are unlikely to interfere in the subsequent determination of antimony by either atomic-absorption spectrophotometry or neutron-activation analysis.

Keywords: *Antimony(III) - antimony(V) separation*; *polyurethane foam*; *water analysis*; *sodium diethyldithiocarbamate*

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Analyst, 1977, **102**, 842–845.

Vessel for Sampling Liquefied Anhydrous Ammonia for Subsequent Trace Oxygen Determination

A vessel for the sampling of anhydrous liquid ammonia in pipelines and storage vessels is described. The apparatus allows a representative sample of the liquid ammonia to be taken and completely vaporised. Complete vaporisation of the liquid sample ensures that the gaseous sample subsequently presented to a laboratory gas chromatograph for analysis is truly representative of the original liquid. By using the gas-chromatographic technique described, oxygen can be determined in the range 0.2–50 p.p.m. *V*/*V*. Hydrogen, nitrogen, argon and carbon monoxide can also be determined on the same chromatogram. Measurements of the oxygen content of ammonia in various storage vessels has allowed an inspection policy for such vessels to be formulated for early detection of stress-corrosion cracking initiated by oxygen. By correct choice of the liquid and expansion volumes of the sample vessel, it could be used for sampling other liquefied gases.

Keywords: *Oxygen determination*; *liquefied anhydrous ammonia*; *sampling vessel*; *gas chromatography*

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The Analyst

Historical Survey of the Uses of Organic Compounds as Reagents in Analytical Chemistry*

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Early developments in the use of organic substances as analytical reagents are reviewed. Natural materials of plant and animal origin were exclusively used as indicators and qualitative reagents in the 17th and 18th centuries, but were gradually superseded by synthetic products as organic chemistry matured during the 19th century. The development of the Griess test for nitrous acid, including Warington's and Ilosvay's modifications, is outlined. The early applications of purified natural products, such as the alkaloids, and of several sulphur-containing compounds are also discussed.

Finally, the classic chelate-forming reagents for metals, such as copper, cobalt, iron and nickel, are viewed in their historical context as contributing to the development of selective and sensitive analytical reagents.

Keywords: *Organic analytical reagents*; *history*

From the vast number of organic compounds now known to man, many have been applied in the diverse fields of medicine, science and technology with highly beneficial results. Before an understanding of the nature and properties of organic compounds was established in the early years of the 19th century, most applications were empirical and involved compounds of natural origin. As organic chemistry developed, the recognition of functional group reactivity led to a more intelligent use of organic compounds, among which was their use as chemical reagents in analytical chemical processes. In more recent years, the work of F. Feigl[1] has provided ample evidence, not only of the inestimable value of organic compounds in chemical analysis, but also of the possibilities for synthesising new and more effective reagents.

Such an important feature of modern analytical chemistry must have had some interesting beginnings and it is the purpose of this survey to draw attention to some of the more significant historical landmarks in the early uses of organic analytical reagents.

The very early history of chemical analysis has been admirably summarised by F. Szabadváry in his comprehensive "History of Analytical Chemistry" (1966).[2] Therein, he describes the wet reaction which Pliny used to establish whether a sample of copper sulphate had been adulterated with iron sulphate or not. Using papyrus soaked in an extract of gall nuts from the oak, *Quercus infectoria*, Pliny described the colour reaction that occurred, and thus originated the technique of spot-testing with an organic reagent (gallotannic acid).[3]

If this is accepted as a valid example, then Pliny's test is all the more remarkable in that a period of some 1 600 years has to elapse before similar analytically-based reactions begin to be described in the writings of scientists and philosophers in the 16th and 17th centuries. Otto Tachenius, Pharmacist and Physician from Westphalia, describes in some detail (1666) the reactions of the aqueous extract of gall nuts with solutions of several metal salts, and thus may be credited with the publication of the first systematic examination of an organic reagent for metal ions.[4]

Such primitive beginnings necessarily involved natural substances, principally those of vegetable origin. Extracts of plants, particularly the flowers, provided solutions of colouring matters for use in the dyeing of clothes. It is not surprising that the colour changes of such solutions in the presence of acids and alkalis attracted the interest of the mediaeval chemists and pharmacists. Robert Boyle provides the best example of an early chemist employing colour reactions as diagnostic tests for various chemical substances and his use of plant and

* Presented at The Chemical Society Annual Chemical Congress, London, March 30th, 1977.

animal extracts, such as syrup of violets, extracts of cornflower, cochineal and litmus, served to introduce these reagents to a wider chemical fraternity. His use of an infusion of *Lignum nephriticum* (a species of pterocarpus) as a test for acids and alkalis is of added interest in that the sky-blue fluorescent colour of the solution is discharged by the addition of acid, thus anticipating the introduction of fluorescent indicators by some 250 years.[5] Boyle's "syrup of violets" became a recognised test for acids and alkalis, and led to its adoption as the first acid - base indicator in what subsequently developed into titrimetric analysis. It is pertinent to look at this development in the present context, because technical analysis and industrial chemistry were growing rapidly in the 18th century, and most new chemistry in those days was analytical chemistry.

In 1767, William Lewis published a short treatise on the quality of American potashes in which he described one of the earliest examples of an exact titrimetric process.[6] He titrated his sample of potash with acid and detected the point of saturation "not by the ceasing of effervescence, which is extremely difficult if not impracticable to hit with tolerable exactness, but by some effect less ambiguous and more strongly marked, such as the change in colour produced in certain vegetable juices, or on paper stained with them. The finer sort of purplish-blue paper used for wrapping sugar in, answers sufficiently well for this purpose." Here is a precise description of pH test paper used in a neutralisation reaction. Ten years later, in 1777, Carl Friedrich Wenzel[7] described the chemical use of litmus, curcuma and brazilin in titrimetric processes and from that time the use of such substances became commonplace in acidimetry and alkalimetry. The development of titrimetry has been discussed by Ranke-Madsen.[8]

The first book in English to treat exclusively the subject of volumetric analysis was by Francis Sutton[9]; the first edition appeared in 1863. Even in the second edition, published in 1871, Sutton describes only three indicators for use in acid - base titrations: litmus, cochineal and turmeric paper. The first synthetic organic compound to be used as an acid - base indicator appears to be phenolphthalein, proposed by E. Luck[10] in 1877, following A. Baeyer's synthesis of this and other phthaleins in 1871. This application was closely followed (1878) by W. von Miller's[11] use of O. Witts' tropaeolin OO and Lunge's observation that several azo dyes, in particular Poirrier's orange III (methyl orange), are unaffected by carbonic acid and are thus ideal indicators for the titration of carbonates with mineral acids (technical analysis of soda).[12] These synthetic organic compounds thus constitute early applications as reagents in chemical analysis.

An interesting note by Greville Williams[13] in 1878 also recommends the "Orange 3 of Poirrier" in place of litmus in volumetric analysis, and it seems that this proposal was made simultaneously and independently of that of G. Lunge.

Nevertheless, such was the impetus that synthetic organic chemistry had given to the development of new compounds with suitable acid - base properties that by the year 1901 a book on the subject by Glaser was published in Wiesbaden.[14] It is worth noting here that methyl red was not introduced as an acid - base indicator into titrimetric analysis until 1908,[15] 30 years after methyl orange.

Surprisingly, an organic compound found very early use in redox titrimetry. The discovery by Berthollet[16] of the bleaching action of aqueous and alkaline solutions of chlorine led to a new technical process for the bleaching of textiles. Control of the strength and quality of the bleaching solution became necessary and a Rouen pharmacist, Francois Antoine Henri Descroizilles,[17] was one of the first to work with Berthollet's solution[18] and to develop a suitable titrimetric procedure for its assay. Sometime in 1787 or 1788, he elaborated what can truly be described as the first redox titration in which a solution of natural indigo in dilute sulphuric acid was "titrated" with a solution of chlorine water or hypochlorite solution. Here the reagent serves as its own indicator, being converted irreversibly to colourless oxidation products. This remarkable process, designed for a specific technical analysis, remained much as Decroizilles described it until Gay-Lussac[19] (1835) introduced a solution of arsenic(III) oxide as the reductant for hypochlorite in which the end-point of the titration was shown by the addition of 1–2 drops of a solution of indigo as the colour indicator. Almost immediately after the publication of Decroizilles' analytical method, James Watt (the engineer), who was among the first in Britain to employ chlorine in bleaching processes, wrote to Berthollet to tell him that he (Watt) preferred a solution of cochineal rather than indigo, particularly when the solution of chlorine was alkaline (that is, hypochlorite).[20] With Gay-Lussac's procedure as a

model it is odd that no synthetic organic compound was used as a redox indicator in titrimetry until Knop's introduction of diphenylamine in 1923.

This brief account of natural products used as specific reagents in these early analytical processes shows that the chemist was well aware of the value of organic reagents in the latter half of the 18th century. However, these were just a trickle compared with the flood which the new organic chemistry of the 19th century was to release. The selection of pertinent examples from this period has been somewhat difficult and some classification of types of reagents is desirable. If one keeps in mind that the main purpose of chemical analysis was to provide quality control in manufacturing processes, then the more commercially important the product of such a process, the more attention its analysis received. Basic chemicals, such as the mineral acids, the alkalis, hypochlorite and metal salts, were all subject to various analytical processes and where organic compounds could be used as reagents, these began to be introduced.

The use of phenol in sulphuric acid was recommended by Sprengel[21] in 1863 as a test for nitrate. It did not differentiate nitrate and nitrite. In the same paper, Sprengel also recommended phenoldisulphonic acid for the detection and determination of nitric acid; the reagent is still in use for this purpose. The detection of nitrate and nitrite in sulphuric acid made use of the oxidation of diphenylamine, a reaction first observed by A. W. Hofmann[22] in 1864, but applied analytically by Kopp[23] in 1872. Credit for this is often ascribed to Lunge (1894).[24] Pyrogallol reacts only with nitrite in dilute sulphuric acid to give a yellow - brown colour. Schönbein[25] used this phenomenon in 1862 to distinguish between nitrate and nitrite. Only in concentrated sulphuric acid does nitrate react with the triphenol. It is of interest to record here that Döbereiner[26] observed the uptake of oxygen by an alkaline pyrogallol solution in 1823, although it was Liebig[27] in 1851 who first made use of the reaction for the quantitative removal of oxygen from air and other mixtures of gases. Other phenolic substances, such as resorcinol and gallic acid, found similar use for the detection of nitrate and nitrite, but perhaps the most significant development around this time was the elaboration of the diazo test for nitrite by Peter Griess in 1878, following his extensive studies of the diazo reaction in the years 1865 onwards. A brief history of this test is worth including in this survey, not only because it provides an early example of a specific (and highly sensitive) test for a particular chemical entity, but because many misconceptions of the test appear in the literature (Fig. 1).

Griess first[28,29] used "diamidobenzoic acid" (1871) but later recommended *m*-phenylenediamine, which he had earlier discovered, as the most suitable reagent (1878). This reagent, on treatment with nitrous acid, rapidly turns into a yellow - brown dye, known as Bismark Brown. Preusse and Tiemann[30] immediately applied this test on a quantitative basis. Although this test remained in use for some years, Griess himself was not entirely satisfied with it, and when Roussin, in 1878, described the red azo derivative obtained by coupling diazotised sulphanilic acid with 1-naphthylamine, Griess was quick to appreciate its potential for the detection of nitrous acid and nitrites. His paper[31] in *Berichte* in 1879 reviewed earlier procedures and recommended the adoption of the sulphanilic acid - α-naphthylamine reagents. The solution containing the nitrite was first acidified with dilute sulphuric acid and then treated with a solution of sulphanilic acid in water. After some minutes, a solution of α-naphthylamine sulphate was added. The presence of nitrous acid was indicated by the formation of a pink to red colouration in the solution.

At this time, in England, the agricultural scientist Robert Warington of the Rothamsted Experimental Farm was interested in the presence of nitrous acid in the atmosphere. He believed that this could best be shown by testing rain water for its presence, or exposing distilled water to the atmosphere for some time and testing the water for nitrite. At the suggestion of O. N. Witt, he discarded the *m*-phenylenediamine test in favour of the new α-naphthylamine reagent because of its extreme sensitivity. He describes Griess' procedure, which he immediately modifies.[32] His description is worth quoting. "In employing this test, I have used exclusively hydrochloric acid and the hydrochloride of naphthylamine. It is apparently unnecessary to wait between the addition of the two reagents, as recommended by Griess, equally good results being obtained by adding them simultaneously." His procedure, in effect, involves adding successively to the test solution (about 10 ml in volume) 1 drop of hydrochloric acid (1 : 4), 1 drop of a nearly saturated solution of sulphanilic acid and 1 drop of a saturated solution of naphthylamine hydrochloride. He goes on to show that the test is sensitive to 1 part of nitrous acid in 1 000 000 000 parts of water, some hundred times more sensitive than the *m*-phenylenediamine reagent. He comments that "the amount of delicacy

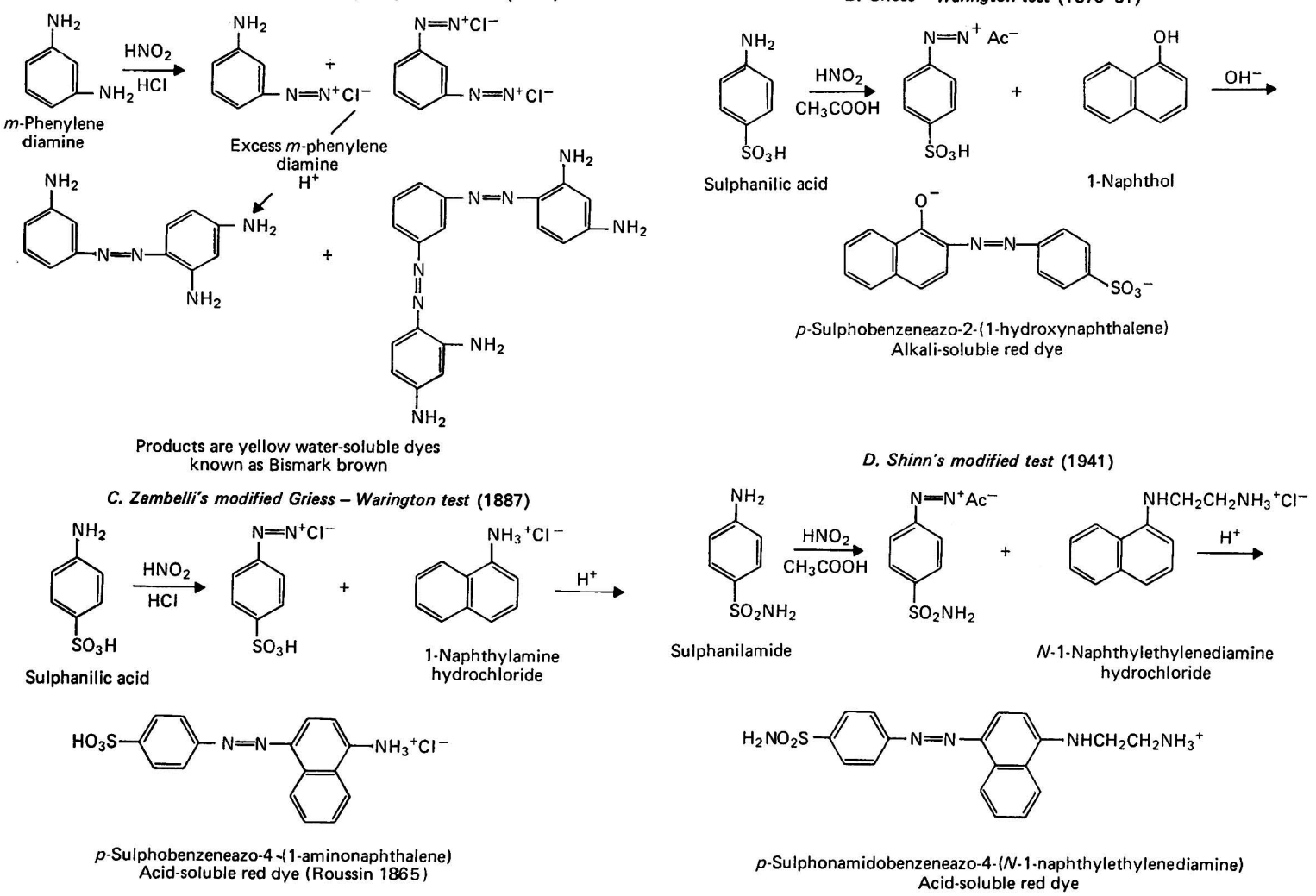


Fig. 1. Chemistry of the Griess tests for nitrous acid.

possessed by the naphthylamine reaction is truly astonishing, and places it in the front rank of all qualitative tests." In the style of the true experimenter, he demonstrated to his audience the presence of nitrous acid in the air of the meeting room of the Chemical Society during the presentation of his paper (sometime between October 1880 and March 1881).

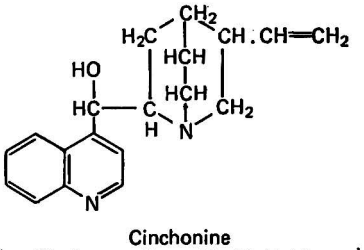
Some 8 years later, Lajos Ilosvay[33] re-examined the Griess - Warington reaction and recommended the replacement of the mineral acid by acetic acid, at the same time showing the use of the test for the nitrate ion following its reduction by metallic zinc. He claimed that greater sensitivity was obtained in an acetic acid medium, a fact corroborated by Weston[34] (1905) in a comparative study of the Griess - Warington and Ilosvay procedures. It was left, however, to Lunge[35,36] (1889–1890) to recommend the final form of the reagent as a mixture of its two component solutions. One important feature of the Griess - Warington test is that its high sensitivity makes it disadvantageous in testing for moderate amounts of nitrite; the excess nitrous acid rapidly destroys the red azo-amino dyestuff. This problem is readily avoided by using Zambelli's modified procedure (1887), in which the α-naphthylamine is replaced by α-naphthol or phenol, and coupling to form the azo dye is effected in an alkaline medium. Zambelli[37] did not claim this advantage for his test, but considered it to be as sensitive (1 part of nitrous acid in 25 000 000 parts of water) as the Griess - Warington test. In fact, the test is conducted in exactly the same way as Griess described for his test, diazotisation of sulphanilic acid followed by the addition of a solution of α-naphthol. Alkali is then added in order to allow the red azo-naphthol dye to form.

These two distinct but similar nitrite tests admirably illustrate how the sensitivity of an identification can be modified by appropriate selection of reaction conditions. Of course, the story does not end here; numerous variations have been described during the ensuing years, culminating in Shinn's replacement of 1-naphthylamine with the more stable *N*-1-naphthylethylenediamine and of sulphanilic acid with sulphanilamide,[38] but the fundamentals of Peter Griess' reaction are still utilised in the detection and determination of nitrite at the trace level in air and water.[39]

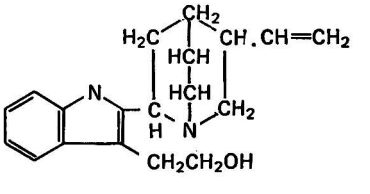
Among the compounds of natural origin, the alkaloid bases have always attracted attention. Their reactions with inorganic substances have added interest in providing possible means for the detection and determination of these physiologically and pharmacologically important substances. Their sparingly soluble salts with, for example, hexachloroplatinum(IV) acid have been known for very many years and numerous microscopical crystal tests have been described for the identification of these alkaloid salts.[40]

However, more direct applications of these organic bases as analytical reagents have occurred and, of these, the use of brucine is of considerable interest. Kersting,[41] in 1863, described the red colour formed when a solution of brucine in concentrated sulphuric acid is treated with nitrate, and recommended the reaction as a qualitative test for nitric acid. Other chemists, notably Lunge[42] (1894–1902) and Winkler[43] (1899–1902), published colorimetric methods, which indicates the extent to which colour was being used in quantitative analyses in the last two decades of the 19th century.

The cinchona alkaloids, particularly quinine and cinchonine (Fig. 2), have proved to be useful analytical reagents. Thus, Lefort[44] in 1881 recommended quinine for the precipitation of tungstic acid and Cremer[45] studied the reaction of cinchonine with tungsten in 1895. This is now the preferred reagent although it was not applied in an analytical procedure until 1918.



Cinchonine
(*ex Cinchona calisaya*, Wedd. Rubiaceae)
and other *Cinchona* species



Cinchonamine
(*ex Remijia purdieana*, Wedd. Rubiaceae)

Fig. 2. *Cinchona* and *Remijia* alkaloids.

The well known orange - red cinchonine iodide - bismuth iodide complex salt was first recommended as a test for bismuth by Leger[46,47] in 1888. The elusive alkaloid, cinchonamine, from *Remijia purdieana* has the most interesting analytical property of forming a sparingly soluble nitrate salt. Arnaud and Padé[48] suggested the use of this compound for the detection and determination of nitrates some 20 years before Busch's discovery in 1905 of the remarkable salt-forming properties of nitron. Howard and co-workers[49,50] (1905–1909) recommended the alkaloid for the gravimetric determination of nitrate. Some confusion over the chemical constitution of this substance exists in the literature. It is, in fact, an indole alkaloid and not a quinoline one like the *cinchona*-based alkaloids (see Fig. 2). Its synthesis and constitution were established by Prelog and his co-workers as late as 1950.[51] It has always been a very rare substance (unlike the quinine alkaloids) and it is not surprising that no other studies of the nitrate precipitation followed Howard's work. Presumably, the introduction of nitron in 1905 provided a more accessible reagent for this and other purposes. An attempt, some years ago, to acquire a sample of cinchonamine from many possible sources throughout the world ended in complete failure, so that no modern assessment of this interesting reagent is possible. In more recent times many other alkaloids have been suggested as analytical reagents, and interested readers are referred to F. Welcher's compendium of organic analytical reagents for further information.[40]

Among the large number of sulphur-containing compounds (Table I), the most significant is rubeanic acid or dithiooxamide. F. Wohler,[52] in 1825, noted the reactions of this compound with metal ions leading to coloured precipitates and observed that iron salts behaved differently.

TABLE I

SOME SULPHUR-CONTAINING COMPOUNDS USED AS ANALYTICAL REAGENTS

Dithiooxamide (rubeanic acid)	$H_2N-C(=S)-C(=S)-NH_2$	F. Wohler (1825) P. Ray and R. M. Ray (1926)
Potassium ethylxanthate	$C_2H_5OC(=S).SK$	M. Sievert (1864)
Ammonium thioacetate	$CH_3C(=O).SNH_4$	R. Schiff and N. Tarugi (1894)
Potassium dithiooxalate	$KO-C(=S)-C(=S)-OK$	H. O. Jones and H. S. Tasker (1909)
Sodium diethyldithiocarbamate	$(C_2H_5)_2N-C(=S)-SNa$	M. Delapine (1908)
Diphenylthiocarbazone (dithizone)	$C_6H_5-NH-NH-C(=S)-N=N-C_6H_5$	H. Fischer (1926)

The modern use of the reagent stems from the work of Ray and Ray (1926).[53] Potassium ethyl xanthate was suggested as a reagent for the detection of nickel in 1878.[54] The yellow colour of the nickel complex is stable in ammonia solution, unlike the situation with the copper complex. Potassium ethyl xanthate is a very useful reagent, particularly for copper and molybdenum. Its introduction as a reagent is a result of the work of Sievert.[55] An interesting, but neglected, reagent is dithiooxalic acid, usually used in the form of its potassium salt, which Jones and Tasker[56] used for the detection and determination of nickel and cobalt. The nickel complex has a magenta colour and can be used to detect as little as 1 part of nickel in 40 000 000 parts of water. This compound was described in 1909, and it is interesting to

speculate that its value as a reagent for nickel was probably diminished by the already established Chugaev reagent dimethylglyoxime; its synthesis is undoubtedly somewhat malodorous.

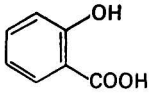
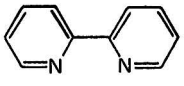
In the 1950s, considerable attention was given to the use of thioacetamide as a ready source of hydrogen sulphide for the precipitation of metal sulphides. This amide is readily hydrolysed by warming in aqueous solution. In 1894, Schiff and Tarugi[57] recommended ammonium thioacetate for just this purpose, particularly to replace hydrogen sulphide gas in systematic qualitative analysis. Little further work appears to have been done with this reagent, although Danziger[58] (1902) recommended the blue cobalt complex obtained by reducing the cobalt thioacetate solution with tin(II) chloride and extracting with amyl alcohol as a highly sensitive test.

As a class of compounds, the dithiocarbamates have proved to be a fruitful field for those searching for new reagents. The best known compound, sodium diethyldithiocarbamate, arose from the work of the French chemist Delepine.[59] His studies of the complexes of various alkyldithiocarbamates led him, in 1908, to propose the diethyl compound for the detection of copper. It is still very much in use today.

The intense red colour that a solution of thioglycolic acid (Table II) gives with iron salts in ammonia solution was used first by Andreasch[60] in 1879. Other workers soon confirmed its value for the detection and determination of iron, its advantages being high sensitivity (1 part of iron in 10 000 000 parts of solution), high colour stability and independence of the original oxidation state of the iron. The reagent acts as its own reducing agent to provide iron(II) for the colour reaction.

TABLE II

ORGANIC REAGENTS FOR IRON

Salicylic acid		A. Vogel (1876)
Thioglycolic acid	$HSCH_2COOH$	R. Andreasch (1879)
Butan-2,4-dione (acetylacetone)	$CH_3.\overset{\overset{O}{\|}}{C}.CH_2.\overset{\overset{O}{\|}}{C}.CH_3$	A. Combes (1888)
2,2′-Bipyridine (α,α′-dipyridyl; 2,2′-bipyridyl)		R. Hill (1930)

Although the term chelate was not applied in coordination chemistry until 1920, when Morgan and Drew's[61] work on metal acetylacetonates led them to coin the adjective for this special type of inner complex compound, the existence of these complexes had been recognised for many years. The work of Werner and of Hantzsch at the turn of the century did much to explain the behaviour of organic compounds with metal ions. The synthesis of the universal complexing agent acetylacetone (Table II), and many of its metal complexes, was described by A. Combes in 1888.[62] He observed the intense red colour that it gave with aqueous iron(III) solutions and suggested that it might be used for the detection of iron. Pulsifer[63] (1904) used acetylacetone for quantitative work and showed it to be superior to thiocyanate, not only in sensitivity but also in colour stability. His paper is of interest in that a bibliography of methods for the detection and determination of iron is appended to his own findings. This bibliography shows that Auguste Vogel[64] is responsible for recommending salicylic acid (Table II) as a reagent for iron; this he did in 1876. The work of Edgar F. Smith,[65] in 1880, indicates that salicylic acid was by then a well established reactant for iron(III) solutions. Although it is barely half as sensitive as thiocyanate, it is preferred when copper is present. Gregory's study[66] of the reagent came somewhat later (1907).

In their pursuit of new compounds, the organic chemists kept a wary eye open for new and unusual reactions of their synthetic products. An excellent example of the rewards that such observations could provide is given by the work of M. Ilinski.[67] His interesting account, in 1884, of the preparation and properties of the nitrosonaphthols ended with a short statement concerning the behaviour of the isomeric nitrosonaphthols with cobalt chloride. The formation of characteristic chlorine-free cobalt complexes with unusual stability towards acids, alkalis, oxidation and reduction prompted an immediate investigation of the usefulness of α-nitroso-β-naphthol as an analytical reagent. Within the space of a few months, Ilinski and van Knorre[68,69] presented an account of the separation of nickel and cobalt in which the conditions for the quantitative precipitation of cobalt were described.

Not all new compounds received such immediate attention as analytical reagents, the classic example being 2,2′-bipyridine (α,α′-dipyridyl; 2,2′-bipyridyl). Although Blau[70] meticulously investigated the metal complexes of this and related heterocyclic compounds (1,10-phenanthroline) and described their highly coloured iron(II) complexes,[71] some 30 years were to elapse before their use in analysis was described. It may be that improved methods of synthesis, particularly by Hein and Retter,[72] led to a renewed interest in these compounds. Hill[73] (1930) must receive priority in the quantitative use of 2,2′-bipyridine (Table II); he also developed his own synthesis of the reagent. His work was followed by Feigl and Hamburg's use of the reagent in a spot-test procedure in 1931.[74] In the same year, Walden, Hammett and Chapman introduced the iron(II) complex as a high potential redox indicator and a fascinating new chapter in the development of organic analytical reagents had begun.

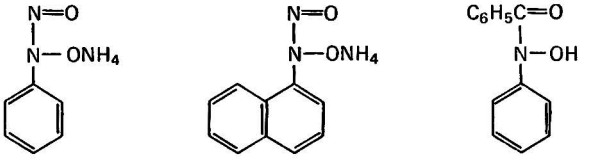
Without doubt, the most significant new reagent of its time was dimethylglyoxime, the subject of a short paper by the Russian chemist Leo Chugaev[75] in 1905, entitled "A New Delicate Reagent for Nickel." Chugaev recorded a sensitivity of 1 part of nickel in 400 000 parts of water for dimethylglyoxime and showed that 0.1 mg of nickel was still detected in 500 mg of cobalt. This analytical work was an offshoot of his extensive studies of the metal complexes of the α-dioximes, and it is interesting to speculate on the element of chance that always seems present in major discoveries, which in this instance led Chugaev to this remarkable reagent. What if dimethylglyoxime had existed in either of its other two possible geometrical isomers, or if the crystal lattice of the solid nickel complex had been the same as that of the copper and cobalt complexes? The reasons for the high sensitivity and selectivity of the nickel reaction were not then known to Chugaev, but this did not prevent him from reporting his experimental observations and introducing one of the most effective reagents into chemical analysis.

In the same year, and in the same journal, Max Busch[76] described the synthesis of certain *endo*-iminotriazoles, one of which possessed an extraordinarily sparingly soluble nitrate. This work was followed in the next paper in the journal[77] by the application of 1,4-diphenyl-*endo*-anilinodihydro-1,2,4-triazole to the gravimetric determination of nitric acid and nitrates. Busch was well organised; he had already coined the much more useful trivial name "nitron" for his new reagent and had arranged with E. Merck to make it commercially available. A similar happy story cannot be told of the organic precipitant for the sulphate ion, which reflects the great difference in the analytical chemistry of the sulphate and nitrate ions. There was no alternative inorganic reagent for determining the nitrate ion equivalent to barium chloride for the sulphate ion. Although Zinin[78] had observed the sparing solubility of benzidine sulphate in 1845, and Vaubel[79] had used this fact in 1896 to determine benzidine gravimetrically, it was Müller[80,81] in 1902 who first used the diamine in order to determine the sulphate ion. His procedure involved the preparation of a standard solution of benzidine hydrochloride, the alkali equivalence of which was known by direct titration in the presence of phenolphthalein. A known volume of this solution was added to the unknown sulphate and the precipitate of benzidine sulphate was filtered off. The filtrate was titrated with standard alkali to determine the unconsumed reagent. Raschig[82] (1903) preferred to filter off the benzidine sulphate, suspend it in water and titrate the free acid formed by hydrolysis of the salt with standard acid. Neither worker ever really accepted the other's procedure; Müller claimed that Raschig's procedure was subject to errors arising from the solubility of benzidine sulphate in water. In spite of this, Raschig's method prevailed and benzidine was gradually accepted and remained the one organic reagent for the sulphate ion until the introduction of 4-amino-4′-chlorobiphenyl[83] in 1952.

The first decade of the 20th century was an active time for the introduction of significant new

reagents. Some have already been mentioned, but there were others of equal importance. In 1900, Cazeneuve[84] proposed the symmetrical diphenylcarbazide for the detection of several metal ions, but particularly chromium in the form of chromate. The reactions of this substance with metal ions seem to have been known for some time; Michael Faraday[85] reported the sensitive nature of these coloured metal carbohydrazides in chemical testing in 1857. The relationship between diphenylcarbazide and diphenylcarbazone is an interesting one. The latter is obtained by oxidation of the former, and many of the reactions of diphenylcarbazide are said to occur as a result of the formation of the carbazone. Cazeneuve[86] studied the behaviour of both substances and believed that the carbazone was the active agent, although Feigl and Lederer[87] did not subscribe to this view. The reactions of diphenylcarbazone with various metal salts were first described by Skinner and Ruhemann[88] in 1888, and its use for the detection of mercury was described by Meniere[89] in 1908. Although diphenylthiocarbazone (dithizone, Table I) was first synthesised by Emil Fischer[90] in 1878, no attempt was made to use this compound in analytical chemistry until 1925. It is of interest to note that Emil Fischer recognised the acidic nature of dithizone and even described the formation and colour of its zinc salt, but it remained for Hellmut Fischer[91] to initiate the investigations of this remarkable substance, which was to become without doubt the foremost reagent for the heavy metal ions and a most significant influence on the development of trace metal analysis.

No account of the history of organic analytical reagents, however brief, can omit mention of the use of ammonium *N*-nitrosophenylhydroxylamine, better known as "cupferron." Introduced by Oskar Baudisch[92] in 1909 as a reagent for the precipitation of iron and copper, its employment in separating iron from aluminium and titanium from chromium are just two examples of the many practical uses to which this compound was put in applied analysis, particularly in the mineralogical and metallurgical fields. The application of cupferron undoubtedly helped to solve many important problems of chemical analysis, but it is worth conjecturing that it also created a complacency amongst chemists that no other organic reagent could better it. How else can one explain the dearth of new reagents proposed in years between 1909 and 1926, when Berg and Hahn independently proposed 8-hydroxyquinoline? The remarkable feature of cupferron is the tenacity of its originator, Dr. Oskar Baudisch, who, at the ACS meeting in Rochester, N.Y., in September, 1937, proposed the naphthyl analogue of his earlier reagent, which he called "neo-cupferron" and demonstrated its improved sensitivity for the precipitation of iron and copper.[93] This work undoubtedly influenced Shome[94] in his introduction of the *N*-benzoyl derivative of phenylhydroxylamine in place of cupferron in 1950 (Fig. 3). Many substituted benzohydroxamic acids have now been recommended, the advantages over cupferron of which are difficult to discern.



Cupferron (ammonium *N*-nitrosophenylhydroxylamine) O. Baudisch (1909)

Neocupferron O. Baudisch (1938)

Benzophenylhydroxamic acid S. C. Shone (1950)

Fig. 3. Cupferron and related compounds.

This account of the early development of organic analytical reagents may, perhaps, help to reinforce the author's long-held view that no-one has, *a priori*, introduced into analytical practice an entirely novel reagent from purely theoretical considerations. Once the behaviour of a compound has been usefully established, it can be a relatively straightforward matter to improve upon its characteristics by judicious alteration of its chemical structure. The development of the subject in recent years has been in this direction.

The literature of the past 150 years abounds with a wealth of interesting empirical observations, not all of which have been usefully applied. It is the reward of perhaps re-discovering and exploiting these observations that has led to many "new" analytical reagents. As

chemistry continues to progress, we can be assured of many more opportunities to write on the fascinating history of organic analytical reagents.

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Application of the Inverse Zeeman Effect to Background Correction in Electrothermal Atomic-absorption Analysis

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An electromagnet was placed around a specially designed graphite furnace atomiser and the Zeeman effect generated in the atomised sample was used to correct for background absorption generated by the matrix. The optical polarisation properties inherent in the Zeeman effect were utilised to generate two signals, one corresponding to the absorption of the atomic line plus background and the other to the background alone. A simple theoretical treatment was developed that related the observed atomic absorbance to a parameter dependent on magnetic flux density. This parameter, designated R in this work, is a function of the atomic line profile and the Zeeman splitting pattern of the line. The purpose-built apparatus incorporated facilities for simultaneous background correction by both the Zeeman and conventional deuterium-arc techniques.

Measurements were made of the dependence of R on magnetic flux density for silver, gold, cadmium, chromium, mercury, potassium, magnesium, manganese, sodium, nickel and lead in the range 2–9 kG. A reduction in the analytical sensitivity of up to 35% arising from the Zeeman correction procedure was found in some instances. Calibration graphs for lead and nickel were linear with concentration change over two orders of magnitude but were found to invert at high concentrations (about 300 μg ml^{-1}). Using the Zeeman correction system, a background absorbance of 2.0 was compensated to better than 0.005, while that achieved by a well adjusted deuterium arc system was approximately 0.02.

Keywords: *Atomic-absorption spectrophotometry*; *electrothermal atomisation*; *background correction*; *Zeeman effect*

Correction for background absorption is necessary in many analyses in which electrothermal atomic-absorption spectrophotometry is used. This correction is preferably carried out simultaneously with the atomisation of the sample. The background absorption can be measured using either a nearby non-resonance line generated by the analytical light source (the two-line method) or an auxiliary continuum light source that measures the mean absorbance of the background over the spectral region defined by the band pass of the monochromator (the deuterium-arc method). It has been pointed out by Massmann[1,2] that the type of background absorption (line, band or continuum) affects the validity of the background correction procedure. For example, a continuum source system will provide accurate correction for a constant continuum background absorption but will over-estimate that due to a line spectrum. Alternative methods of background correction based on the Zeeman effect overcome some of these problems.

The use of the Zeeman effect was proposed at least as early as 1969,[3] since when several applications have been reported.[4–9] In most instances the Zeeman effect was produced by placing the light source in a magnetic flux which splits the spectral lines to produce additional non-absorbable lines at wavelengths outside the absorption profile of the atomic vapour. Conventional hollow-cathode lamps will not operate satisfactorily in strong magnetic fluxes, and therefore r.f.-excited electrodeless discharge lamps or special atomic light sources have been necessary.[3,5–7] Alternatively, the magnetic flux can be applied to the atomic vapour and the inverse Zeeman effect[10] so generated can be utilised for background correction. Uchida and Hattori[8] applied a modulated magnetic flux to a flame and Dawson *et al.*[9] applied a steady flux to an electrothermally generated atomic vapour. By time sharing or spatial separation of the Zeeman-shifted components with those still under the absorption profile,

either by modulating the magnetic flux or by utilising the polarisation properties inherent in the Zeeman effect, it is possible to correct for background absorption. It is not practicable to generate strong, high frequency modulated magnetic fluxes owing to the inductance of the magnetisation coils, and therefore in the work to be reported in this paper the Zeeman components were identified by their characteristic polarisations. A magnetic flux was applied to the atomic vapour generated by a carbon rod electrothermal atomiser. Preliminary studies[9] showed that several advantages derive from this approach: (1) special lamps capable of operating in strong magnetic fluxes are unnecessary; (2) adequate magnetic fluxes can be generated by small electromagnets; and (3) the correction is carried out at exactly the wavelength of the atomic absorption. In addition, in common with all Zeeman-effect methods, only one light source is used to provide background correction while correction for changes in intensity of the light source is obtained without a complex, double-beam optical system. In this paper, we shall describe an electrothermal atomiser designed for the study of the inverse Zeeman effect in atomised samples and present results on silver, gold, cadmium, chromium, copper, mercury, potassium, magnesium, manganese, sodium, nickel and lead. In addition, the effectiveness of background correction using the Zeeman effect will be compared with that achieved using a deuterium-arc system.

Theoretical

The absorption of atomic resonance radiation in the presence of background absorbing species, assuming that no interactions occur in the vapour, is governed by the expression

$$A_{\lambda T} = A_{\lambda A} + A_{\lambda B} \qquad (1)$$

where $A_{\lambda T}$ is the total absorbance and $A_{\lambda A}$ and $A_{\lambda B}$ are the atomic and background absorbances, respectively, at the wavelength λ. To determine the true atomic absorbance, it is necessary to measure and subtract from the total absorbance that due to the background. This background absorbance measurement, usually made at a wavelength close to that of the analytical line, gives a second absorbance equation:

$$A'_{\lambda T} = A'_{\lambda A} + A'_{\lambda B} \qquad (2)$$

The output signal, S, is obtained by subtracting $A'_{\lambda T}$ from $A_{\lambda T}$:

$$S = (A_{\lambda T} - A'_{\lambda T}) = (A_{\lambda A} - A'_{\lambda A}) + (A_{\lambda B} - A'_{\lambda B}) \qquad (3)$$

If the absorptions of the resonance and reference radiation by the background are identical, *i.e.*, $A_{\lambda B} = A'_{\lambda B}$, an output exactly corrected for background absorption is obtained:

$$S = (A_{\lambda A} - A'_{\lambda A}) \qquad (4)$$

If $A_{\lambda B} - A'_{\lambda B} \neq 0$ there is an error that increases with background absorbance.

The accuracy of the background correction obtained by using a given technique depends greatly on the type of background absorption encountered. The deuterium-arc method will correct adequately provided that the background absorption remains reasonably constant over the monochromator band-width. If this condition does not hold, *e.g.*, if the background is largely due to molecular-band absorption, errors will be introduced because $A_{\lambda B}$ can no longer be equated with $A'_{\lambda B}$. A similar argument can be applied to the two-line method.

Equation (4) can be re-written as

$$S = A_{\lambda A}(1 - R) \qquad (5)$$

where R, the absorbance ratio, is defined as

$$R = \frac{A'_{\lambda A}}{A_{\lambda A}} \qquad (6)$$

R is the fractional reduction in sensitivity arising from use of the background-correction procedure, assuming $A_{\lambda A}$ to be unaffected by changes in the magnetic flux. In both the deuterium-arc and two-line methods of background correction, R is effectively zero because the atomic absorption of the reference radiation is very small compared with that of the resonance radiation. It will be shown later that this is generally not the case in Zeeman-effect background correction, where the R values are of great importance.

In order to correct adequately for most types of background absorption, the background-absorption measurement should be made at the same wavelength as that of the resonance line. By using the Zeeman splitting of the resonance lines of the analytical atomic vapour, this requirement can be met, as will be described below.

The observed Zeeman effect, *i.e.*, the symmetrical splitting of the spectral lines emitted or absorbed by an atom in a magnetic flux into a number of polarised components, is a consequence of electronic transitions between Zeeman-split atomic energy levels. For electric dipole radiation, transitions between the Zeeman components of level W and those of level W′ follow the selection rules

$$\Delta J = 0, \pm 1 \; (J = 0 \nleftrightarrow J' = 0)$$
$$\Delta m_J = 0, \pm 1 \; (m_J = 0 \nleftrightarrow m'_J = 0 \text{ if } \Delta J = 0)$$

where J is the angular momentum quantum number and $m_J = J, J - 1, \ldots, -J$ is the magnetic quantum number.

In the totality of transitions that form the Zeeman splitting pattern of any spectral line, there is a tendency for the $\Delta m_J = 0$ components to lie closer to the original wavelength than do those of $\Delta m_J = \pm 1$. The Zeeman components arising from transitions $\Delta m_J = 0$ are known as π-components, while those due to $\Delta m_J = \pm 1$ transitions are called σ-components.

When observed from a specified direction relative to that of the magnetic flux, the π- and σ-components of a Zeeman-split spectral line show markedly different polarisation properties determined by the angle of observation. From a direction parallel to the magnetic flux, the π-components do not appear at all and the σ-components are circularly polarised about the direction of the flux; when observed at right-angles to the magnetic flux, the π-components are linearly polarised in the direction of the flux while the σ-components are polarised perpendicular to the flux. In this work, all observations were made at right-angles to the direction of the magnetic flux, and therefore the wings of a Zeeman-split atomic line appear polarised perpendicular to the flux while the line centre is polarised predominantly parallel to the direction of the flux.

By applying a transverse magnetic flux of sufficient magnitude (several kilogauss) to the analytical atomic vapour, the σ-components of the absorption line can be largely shifted beyond the profile of the resonance line from the hollow-cathode lamp, leaving only the π-components to absorb the resonance radiation. A situation is thus created in which, because of the polarisation properties of these components, resonance radiation polarised parallel to the magnetic flux is strongly absorbed ($A_{\lambda\parallel}$) while the absorbance of that polarised perpendicular to the flux ($A_{\lambda\perp}$) is determined by the flux density and may not be measurable if the latter is sufficiently high. Generally, however, both components will be absorbed equally by the background. It is therefore possible to use the component of resonance radiation polarised parallel to the magnetic flux to measure the atomic absorption plus background and that polarised perpendicular to the flux to measure principally the background absorption.

Equation (4) can therefore be re-written as

$$S = (A_{\lambda\parallel} - A_{\lambda\perp}) \quad \ldots \quad (7)$$

and equation (5) becomes

$$S = A_{\lambda\parallel}(1 - R) \quad \ldots \quad (8)$$

with R given by

$$R = \frac{A_{\lambda\perp}}{A_{\lambda\parallel}} \quad \ldots \quad (9)$$

In Zeeman-effect background correction, R generally varies from unity at zero magnetic flux density to zero at fairly high flux densities; values of R greater than unity may arise in certain circumstances. Maximum analytical sensitivity is obtained when R is at a minimum. The value of R for a given resonance line at a given flux density depends on its Zeeman splitting pattern, line width, hyperfine structure and/or isotope shift; in addition, external factors such as lamp current and the concentration of analyte atoms present in the atomiser play a part. By plotting the ratio, R, against magnetic flux density, valuable information can be obtained concerning the applicability of Zeeman-effect background

correction to the analyte in question. The prediction of sensitivity loss from the R curves is most accurate when a so-called "normal" Zeeman pattern is encountered, *i.e.*, one in which the line splits into three components, one (unshifted) π-component and two equally displaced σ-components, because in this instance the absorbance of parallel-polarised resonance radiation, $A_{\lambda\parallel}$, is independent of the magnetic flux density. For most resonance lines, however, the absorbance of such resonance radiation is slightly reduced by the magnetic flux density because most lines exhibit the so-called "anomalous" Zeeman effect, in which large numbers of π- and σ-components may be generated and the π-components are also shifted from the central wavelength. The shape of the R curves will be discussed further when the experimental results are presented.

Experimental

The two fundamental considerations on which the design of the Zeeman-effect atomic-absorption spectrophotometer was based were that the Zeeman splitting should be produced in the analytical atomic vapour generated by an electrothermal atomiser and that the separation of the resonance absorption and the reference signals should be effected by utilising their mutually perpendicular polarisations. The former requirement was met by the construction of a special-purpose atomiser, but the latter was more difficult to meet owing to the limited transmission of ultraviolet radiation by most polarising materials. Two systems were assembled, in one polarising filters transparent down to about 270 nm being mounted in the apertures of a light-chopper disc interposed in the optical path from the hollow-cathode lamp, and in the other a Glan polarising prism transparent down to 220 nm being used to separate the radiation, after passage through the monochromator, into two components of mutually perpendicular polarisation measured by separate photomultipliers. In both systems linear gating circuits were incorporated in the electronic systems for the separation of the several optical signals.

Magnet and Atomiser

Magnetic flux densities of up to 10 kG are required in order to produce readily detectable Zeeman splitting of the energy levels of most atoms. Such flux densities can be generated by either permanent magnets or electromagnets. As it was desired to study the effect of magnetic flux density on the Zeeman background-correction system, an electromagnet was used. To accommodate an electrothermal atomiser, a uniform magnetic flux was required over a volume of about 1 cm^3. The magnet chosen was a Newport Instruments Type C, driven by a home-made d.c. power supply giving up to 2 A at 260 V with an on-load a.c. ripple of about 0.15%. A Newport Instruments, Type M, Hall-effect probe magnetometer was used to calibrate the magnetic flux density in the atomising space in terms of the current through the coils of the magnet.

There were four principal constraints on the design of the atomiser: (1) the atomisation region should be sufficiently small to be contained within the 1-cm^3 effective volume of the magnetic field; (2) it must be robust enough to withstand the forces (about 2 N) acting on it when carrying large currents (about 200 A) at a strong magnetic flux density (up to 10 kG); (3) it should operated in an inert atmosphere; and (4) optical alignment and sample injection should be simple. The construction of the atomiser is shown in Fig. 1(*a*). The graphite rods shown in Fig. 1(*b*) were made from 6.15 mm diameter Ringsdorff, grade RWO, spectrographic graphite. The sample (2 μl) was dispensed with an SMI micro-pipette down a 2.5 mm diameter longitudinal hole in the rod and the atomic vapour was viewed through a transverse hole 2.5 mm in diameter. The longitudinal hole also served as a "chimney" for the escape of smoke generated during ashing of the sample. Expansion and contraction of the rod were accommodated by means of a sliding joint in the rod holder. The rod holders were cooled with water and connected in series with the return flow of the magnet cooling system. The power supply for the atomiser was built in-house and was capable of providing up to 12 V, 300 A in four programmable steps.

Several shapes of rod were investigated with a view to obtaining analytical sensitivity and reproducibility suitable for studies of the Zeeman effect. For the shape shown in Fig. 1(*b*), under typical conditions, samples were dried by passing a current of 15 A for 15 s. Ashing required a current of 50 A for 30 s followed by atomisation at 170 A for 8 s. After flash-

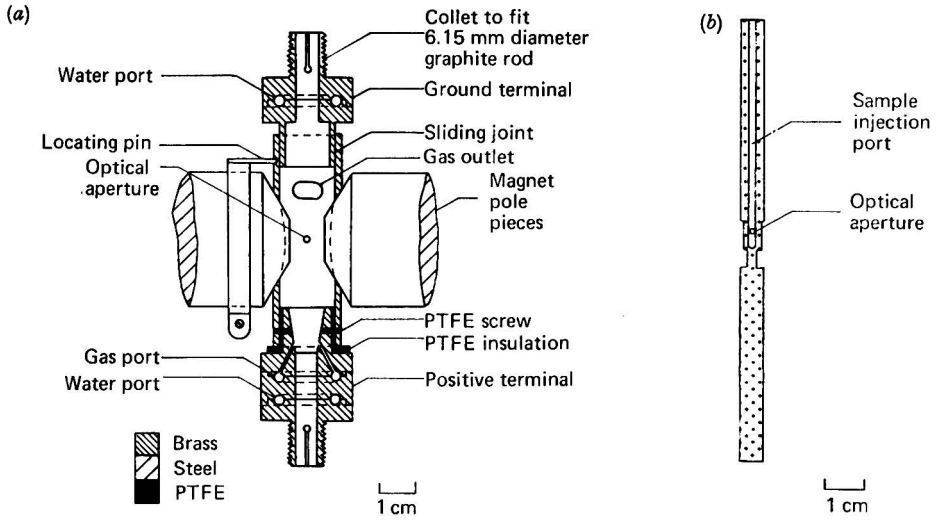

Fig. 1. Vertical furnace atomiser: (*a*), cross-section of atomiser; and (*b*), cross-section of graphite furnace tube. The optical axis is at right-angles to the plane of the diagram.

cleaning at 220 A for 3 s, the furnace was allowed to cool for 60 s before injection of the next sample.

A maximum temperature of 2 600 °C was obtainable; owing to the mass of graphite in the system the heating rate was slow (about 300 °C s^{-1}) and hence the atomisation peaks were broad compared with those generated by more usual electrothermal systems with faster heating rates (about 1 000 °C s^{-1}). The sensitivities obtained (Table I) are 1–2 orders of magnitude worse than those of the somewhat comparable mini-Massmann furnace. The reduced sensitivity and increased response time tended to become more pronounced as the

TABLE I

COMPARATIVE SENSITIVITIES OF THE VERTICAL TUBE ATOMISER AND THE MINI-MASSMAN (VARIAN TECHTRON CRA-90 DATA SHEET)

		Sensitivity/pg for 1% absorption	
Element	Wavelength/nm	Vertical tube atomiser	Mini-Massmann
Ag	328.1	5.8	0.2
Au	242.8	240	10.0
Cd	228.8	1.6	0.15
	326.1	210	—
Cr	357.9	160	5.0
Cu	324.8	27	4.0
	327.4	110	—
Hg	253.7	—	60.0
K	766.5	13	1.0
Mg	285.2	0.8	0.06
Mn	279.5	23	0.6
Na	589.0	1.8	0.2
Ni	232.0	380	10.0
	298.3	730	—
Pb	283.3	440	14.0

atomisation temperature of the analyte element increased. Although no special steps were taken to obtain precise results, the reproducibility achieved (relative standard deviation = 0.03) is comparable with that generally reported. In our work, the more volatile metals gave the least reproducible results. As many of the measurements made in the course of this study were expressed as ratios, minor variations in the response of the system were automatically corrected. The lifetime of a graphite rod was more than 250 cycles without detectable deterioration of performance. Such a lifetime was adequate for the completion of a series of measurements on several elements, thereby facilitating the comparison of one element with another.

Optics

The layouts of the two systems used are shown in Fig. 2. The first system [Fig. 2(*a*)] was designed for the examination of the effect of different splitting patterns and line structure on the Zeeman-effect background correction. This system used dichroic polarisers (Polaroid, Type HNP'B, transparent down to 270 nm) mounted in the apertures of a 30-slot chopper disc. The axes of the polarisers were alternately parallel and perpendicular to the disc radii. The disc was positioned so that light from the d.c.-operated hollow-cathode lamp passing through it into the atomiser was polarised alternately parallel and perpendicular to the direction of the magnetic flux. The effective modulation frequency (one cycle consisting of parallel polarisation - blank - perpendicular polarisation - blank) was 750 Hz. Gating signals were generated optically by means of a set of holes at the periphery of the disc and used to gate the photomultiplier signals electronically into three separate channels corresponding to parallel and perpendicular polarisations and furnace-emission continuum.

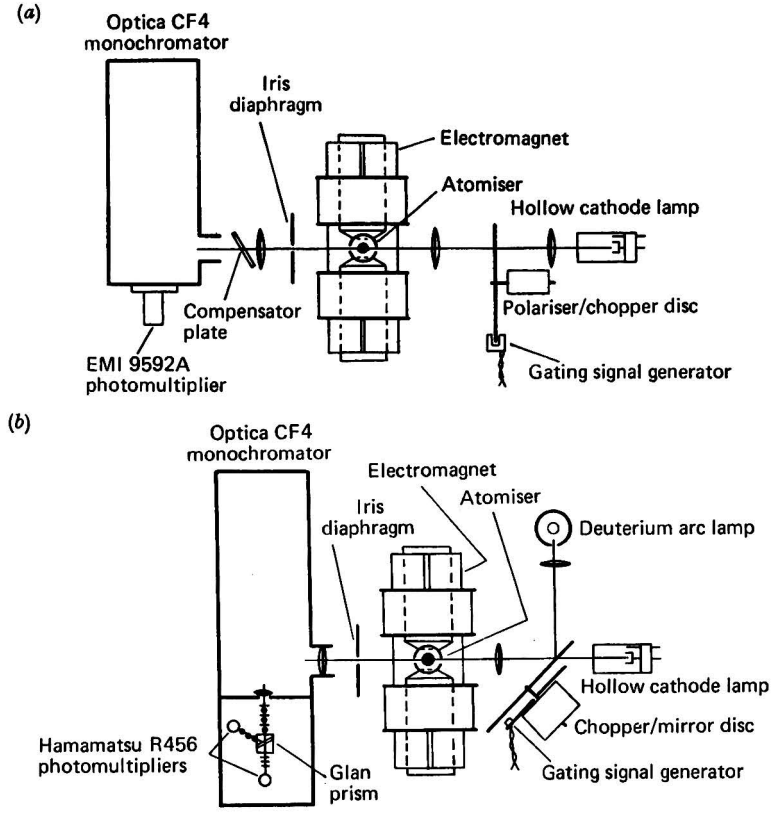

Fig. 2. Schematic diagram of the optics of the Zeeman-effect atomic-absorption spectrometers: (*a*), dichroic polariser system; and (*b*), prism polariser system.

Optical components may modify the polarisation of light incident upon them, particularly if they are made of optically anisotropic materials such as crystalline quartz, and therefore care was taken to check for such effects. In order that the signals of both polarisations should be of similar intensity, a silica compensator was mounted in front of the monochromator entrance slit; by appropriate tilting of this plate the stronger signal could be attenuated.

The second optical system [Fig. 2(*b*)] was developed in order to extend measurements further into the ultraviolet region and to compare the performance of Zeeman background correction with that achievable by a conventional deuterium-arc system. A Glan prism (Rofin Ltd.) capable of transmitting down to 220 nm was mounted behind the exit slit of the monochromator. This prism produced two beams of radiation at approximately right-angles to each other and whose planes of polarisation were also mutually perpendicular. By this means, the intensity of the parallel- and perpendicular-polarised components could be measured simultaneously. A rotating mirror - chopper disc was used to pass light alternatively from the deuterium arc and the hollow-cathode lamp into the system. Magnets attached to the chopper disc induced transient e.m.f.s in a pick-up coil, which provided reference signals for gating of the electronic circuits. An optical compensator plate was not necessary as the amplitudes of the signals were equalised electronically.

Electronics

The special-purpose electronic systems for the two optical configurations were similar and were based on identical d.c.-coupled gating circuits incorporating Intersil IH 5040 linear gates. The first system was designed to isolate the signals corresponding to the parallel and perpendicular polarisations of the resonance radiation and to subtract the background emission of the furnace from each. Three signals were recorded on potentiometric chart recorders (full-scale deflection in 0.4 s): (1) the intensity of the parallel-polarised component of the resonance line ($I_{\parallel}$); (2) the intensity of the perpendicular-polarised component ($I_{\perp}$); and (3) the difference between the two components ($I_{\perp} - I_{\parallel}$). The electronic circuits required for the second optical system were more complex in order that the extra signals generated by the deuterium arc and two photomultipliers could be accommodated. After subtraction of the furnace-emission background, five output signals were available: (1) the intensity of the parallel-polarised components of the resonance line ($I_{\parallel}$); (2) the intensity of the perpendicular-polarised component ($I_{\perp}$); (3) the intensity of the deuterium arc polarised parallel to the field ($D_{2\parallel}$); (4) the difference signals $I_{\perp} - I_{\parallel}$; and (5) the difference signal $D_{2\parallel} - I_{\parallel}$.

Observations and Discussion

The optical systems were aligned so as to obtain maximum signals for both polarisations of the emission from the hollow-cathode lamps and the deuterium arc. The electronic systems were adjusted so as to ensure correct timing of the gating circuits, precise subtraction of the furnace-emission signal and equal signals from the parallel and perpendicular components of the resonance line and the deuterium lamp. The chart-recorded signals were measured with a ruler from a base line corresponding to 100% absorption. The linearity of the system response was verified and for the calculation of results it was assumed that the displacement, D, was proportional to the light intensity, I, hence $A = \log (I_0/I) = \log (D_0/D)$, etc., where D_0 is the initial signal and D is the signal when absorption takes place.

To facilitate comparison of the performance of the Zeeman-effect atomic-absorption spectrophotometer with that of conventional instruments, the absorbance of the parallel-polarised component, $A_{\lambda\parallel}$, was measured. This absorbance approximates to that measured by conventional instruments.

Effects Dependent on Magnetic Flux

The R value as defined under Theoretical gives an indication of the likely usefulness of the Zeeman effect for background correction for a given element; the smaller R is, the more satisfactory the correction is likely to be. This parameter was measured for elements using magnetic flux densities up to 9.2 kG. All observations are recorded in Table II and graphs illustrating the variety of curves obtained are shown in Figs. 3, 4 and 5. These graphs will now be considered and possible explanations of their shapes suggested.

TABLE II

DEPENDENCE OF R VALUES ON MAGNETIC FLUX DENSITY

Element	Wavelength/ nm	Lamp supplier	Lamp current/ mA	Sample concentration*/ $\mu g\ ml^{-1}$	Absorbance	R values: Magnetic flux density/kG 0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0
Ag	328.1	Cathodeon	6	0.1	0.148	1.00	0.76	0.46	0.32	0.16	0.12	0.13	0.10	0.08	0.05
Au	242.8	Perkin-Elmer	8	5	0.189	1.00	0.89	0.83	0.75	0.59	0.41	0.30	0.18	0.14	0.06
Cd	228.8	Perkin-Elmer	8	0.05	0.269	1.00	0.99	0.92	0.87	0.76	0.64	0.55	0.41	0.28	0.24
Cd	326.1	Perkin-Elmer	8	5.0	0.214	1.00	0.49	0.25	0.15	0.08	0.03	0	0	0	0
Cr	357.9	Fivre	15	5.0	0.270	1.00	0.54	0.19	0.12	0.11	0.07	0.02	0	0	0
Cu	324.8	Perkin-Elmer	5	1	0.316	1.00	0.59	0.48	0.48	0.59	0.61	0.57	0.49	0.39	0.29
			10	1	0.232	1.00	0.70	0.52	0.52	0.63	0.66	0.61	0.53	0.42	0.32
			23	1	0.175	1.00	0.77	0.59	0.58	0.73	0.72	0.67	0.60	0.48	0.36
Cu	327.4	Perkin-Elmer	23	3	0.224	1.00	0.92	0.82	0.71	0.58	0.55	0.53	0.49	0.40	0.28
Hg	253.7	Instrumentation Laboratory	7	—	0.201	1.00	0.96	0.94	0.85	0.80	0.72	0.63	0.53	0.43	0.35
K	766.5	Philips (discharge lamp)	612	0.3	0.193	1.00	1.04	1.03	1.01	0.85	0.67	0.50	0.36	0.30	0.21
Mg	285.2	Perkin-Elmer	5	0.02	0.216	1.00	0.96	0.84	0.66	0.53	0.41	0.30	0.22	0.18	0.14
			10	0.02	0.164	1.00	1.00	0.94	0.82	0.67	0.56	0.45	0.33	0.24	0.21
			20	0.02	0.057	1.00	1.07	1.16	1.16	1.09	0.97	0.82	0.67	0.56	0.42
Mn	279.5	Fivre	20	0.5	0.196	1.00	0.87	0.56	0.41	0.29	0.23	0.17	0.13	0.11	0.10
Na	589.0	Philips (discharge lamp)	657	0.05	0.247	1.00	0.87	0.59	0.42	0.29	0.28	0.25	0.17	0.17	0.15
Ni	232.0	Pye Unicam	10	10	0.231	1.00	0.82	0.68	0.49	0.31	0.21	0.11	0	0	0
Ni	298.3	Pye Unicam	10	10	0.122	1.00	0.83	0.62	0.35	0.21	0.16	0.13	0.10	0.09	0.09
Pb	283.3	Perkin-Elmer	10	10	0.200	1.00	0.91	0.76	0.58	0.45	0.32	0.27	0.22	0.20	0.17

* 2-μl samples.

The responses of the two optical systems [Fig. 2(*a*) and (*b*)] were compared using copper (324.8 nm) and lead (283.3 nm). The R values obtained with the two systems were identical to within 0.01 at all magnetic flux densities. The system in Fig. 2(*a*) was the most convenient to use but had a limited response to ultraviolet radiation and no facility for deuterium-arc measurements, and therefore measurements were made with both systems but, as there is no difference between the results produced, no distinction will be made in presenting the results in this paper.

The R curves obtained for the 232.0-nm nickel resonance line and the 283.3-nm lead resonance line (Fig. 3) are typical of those obtained from most of the spectral lines studied. The nickel curve falls rapidly with increasing magnetic flux density, reaching zero at about 7 kG, while that of lead shows a less rapid fall but nevertheless reaches a value of about 0.2 at a flux density of 8 kG. All of the resonance lines examined gave R values of less than 0.4

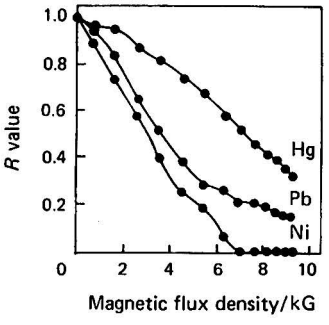

Fig. 3. Dependence of the R value on magnetic flux density for nickel (232.0 nm), lead (283.3 nm) and mercury (253.7 nm).

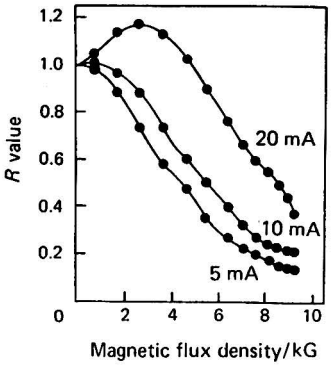

Fig. 4. Effect of lamp current on the R curves of magnesium (285.2 nm).

at the latter flux density, but only nickel (232.0 nm), chromium (357.9 nm) and cadmium (326.1 nm) actually reached $R = 0$. The loss of analytical sensitivity encountered from the worst case examined, that of mercury (253.7 nm) (Fig. 3), is about 35% at 9 kG. This is predictable from the R curve equation (8). For none of the elements studied was the reduction in absorbance of the π-components, $A_{\lambda\parallel}$, greater than about 15% over the magnetic flux density range 0–9 kG, so that the expression $(1-R)$ can be used as a guide to the expected sensitivity loss.

The effect of the lamp current on the R curve for the 285.3-nm magnesium resonance line, which is particularly prone to broadening and reversal, is shown in Fig. 4. The upper curve, corresponding to a lamp current of 20 mA, shows an initial rise to an R value of 1.18 at a magnetic flux density of 2.6 kG. This anomalous behaviour can be attributed to strong self-reversal of the resonance line in the hollow-cathode lamp, yielding a lamp line that is significantly wider than the unsplit absorption line. As the magnetic flux density is increased, the absorbing σ-components are shifted from the relatively low-intensity centre of the reversed lamp line to the higher intensity wings, while the π-components remain unshifted. Thus, assuming that the light emitted from the source is not polarised to any significant extent, it will be more strongly absorbed by the σ- than the π-components, resulting in a value of R greater than unity. The effect of this is to make the output signal, given by equations (7) and (8), negative because in these circumstances the reference signal is more strongly absorbed by the atomic vapour than is the resonance signal. A further increase in magnetic flux density shifts the σ-components beyond the high-intensity parts of the wings and the R curve then falls with increasing magnetic flux density in the usual way. The R curves at lamp currents of 5 and 10 mA are of more usual shape, although the 10-mA curve still exhibits signs of slight self-reversal ($R>1$).

The changes in R with lamp current at constant magnetic flux density lead to changes in analytical sensitivity which are compounded through equation (8) with the usual lamp-current dependence of the absorption $A_{\lambda\parallel}$ and thereby increases the current dependence

of the sensitivity. This effect is illustrated for magnesium in Table III, from which it can be seen that over a lamp current range of 5–20 mA the sensitivity of conventional atomic-absorption varies by a factor of about 4.7 while that of Zeeman-effect atomic-absorption spectrophotometry varies by about 72-fold at 5 kG and about 5.7-fold at 9 kG. The detection limits (Table III) obtained under the same conditions are less affected by changes in lamp current. The best results from the Zeeman system are approximately 20% worse than the corresponding conventionally obtained value.

TABLE III

EFFECT OF LAMP CURRENT AND MAGNETIC FLUX DENSITY ON THE SENSITIVITY AND DETECTION LIMIT FOR MAGNESIUM

	Lamp current/mA					
	5		10		20	
Magnetic flux density/kG	Sensitivity/ pg*	Detection limit/pg	Sensitivity/ pg*	Detection limit/pg	Sensitivity/ pg*	Detection limit/pg
0	0.81	0.18	1.1	0.12	3.1	0.18
5	1.4	0.30	2.4	0.27	100	5.9
9	0.93	0.20	1.4	0.15	5.3	0.31

* Mass for 1% absorption.

Apart from line-broadening influences, the shape of the *R* curves is principally determined by the interaction of the Zeeman splitting with the structure of the resonance line. The resonance lines of most elements have some degree of structure due to hyperfine and isotope shifts, although the number and separation of the lines that form this structure can vary markedly from one resonance line to another, as can the Zeeman splitting pattern. The best example of the effect of line structure encountered in this work was in the *R* curve of the 324.8-nm line of copper, shown in Fig. 5. This curve exhibits a minimum at about 2.5 kG and a maximum at 4.3 kG before falling in the usual manner at higher magnetic flux densities. Measurements at lamp currents of 5 and 23 mA showed a regular difference in which the 5-mA curve lay 15–20% lower at all points over the range measured. A high-resolution scan of this line by Wagenaar and de Galan[11] has shown that it consists of two groups of closely spaced hyperfine components separated by an isotope shift of about 0.4 cm^{-1} ($\equiv$0.0042 nm).

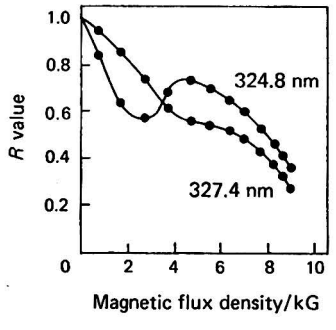

Fig. 5. Effect of line structure and splitting pattern on the *R* curves of the copper resonance lines at 324.8 and 327.4 nm.

Each group of hyperfine components splits independently of the other to give the initial fall observed in the *R* curve. At magnetic flux densities greater than about 2.5 kG, however, some of the furthest shifted σ-components of one group begin to enter the profile of the other group, as emitted by the lamp. This results in an increase in atomic absorption of the reference radiation and to a consequent increase in *R*. As the flux density is increased

further, the R curve begins to fall again owing to the shift of other σ-components away from the centres of their groups. It is of interest that the 327.4-nm copper resonance line, which has a similar structure[12] but a different Zeeman splitting pattern, exhibits only an inflection, rather than a maximum, at about 4 kG. The contrast in the behaviour of the R curves of these two lines illustrates the difficulty of extrapolating from the behaviour of one line to that of another. In the more general case, where the hyperfine- and isotope-shifted components are not so well resolved, the effect of line structure is to broaden the R curve. This is illustrated in the R curve of the 253.7-nm mercury resonance line (Fig. 3), which is known to have a complex structure, albeit poorly resolved at atmospheric pressure.

Calibration Graphs

The Zeeman-effect Glan prism instrument was used to obtain calibration graphs for lead (Fig. 6) and nickel. As concentration ranges of three orders of magnitude were used, the results are presented as a log - log plot, and hence a linear relationship between concentration and absorbance is represented by a straight line of unit gradient; the separation between graphs is determined by differences in sensitivity. For both elements at a low magnetic flux density (about 2 kG) the sensitivities were much less than those obtained in the conventional atomic-absorption mode. As the magnetic flux density was increased, the sensitivities improved until, with nickel at 8 kG, it was virtually the same as in the conventional system. This improvement corresponds to the decrease in R with increasing magnetic flux.

The most striking feature of the calibration graphs is that, at high concentrations and relatively low magnetic flux densities, the slope of the response curve becomes negative. The effect may be understood by reference to Fig. 7, where the absorbance graphs for the parallel- and perpendicular-polarised components of the lead line are presented separately. According to equation (7), the observed signal, S, is equal to the differences between these two absorbances. The onset of curvature of the parallel-polarised component response graph occurs at a lower concentration than that of the perpendicular-polarised components. The difference signal, therefore, after an initial linear dependence on concentration, will increase less rapidly and ultimately will decrease with increasing concentration. If a sufficiently large magnetic flux density is used such that the σ-components of the absorption line are moved entirely outside the lamp profile, then the absorbance of the perpendicular polarised component is zero and the curvature of the calibration graph will be similar to that from a normal atomic-absorption instrument; this condition was achieved with nickel at 8 kG.

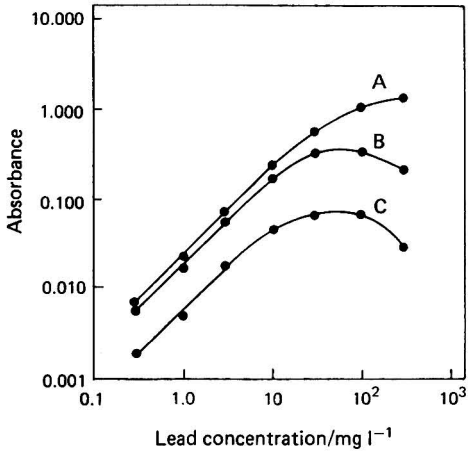

Fig. 6. Calibration graphs for lead (283.3 nm) using the Zeeman-effect spectrometer: curve A, conventional operation ($A_{\lambda\parallel}$, 0 kG); curve B, operation at 8 kG; and curve C, operation at 2 kG.

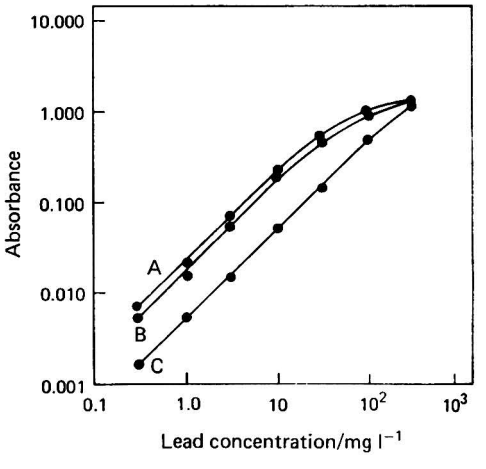

Fig. 7. Dependence on lead concentration of the absorbance of polarised resonance radiation at 283.3 nm: curve A, parallel polarisation ($A_{\lambda\parallel}$), 0, 2 and 8 kG; curve B, perpendicular polarisation ($A_{\lambda\perp}$), 2 kG; and curve C, perpendicular polarisation ($A_{\lambda\perp}$), 8 kG.

The origin of the curvature of the absorbance graphs for the perpendicular- and parallel-polarised light may be either instrumental, arising from, for example, stray light, or fundamental, attributable to the emission- and absorption-line profiles. In the event of the former, a stray light level of the order of 10% would be necessary to produce the observed effect, which is much greater than that detected in the instrument (<1%). Curvature of a fundamental type arises when the atomic-absorption coefficient varies with wavelength over the width of the resonance line emitted by the source. This phenomenon has been investigated in depth by Wagenaar.[12]

The practical problem presented by the turning over of the calibration graphs is the risk of misreporting a high concentration as being much lower, or *vice versa*. This difficulty can be overcome by the use of a sufficiently strong magnetic flux density to ensure that $R = 0$. In practice, this condition may not be achievable for all elements, and an alternative safeguard is the monitoring of the output of the instrument on a fast-response chart recorder, when the difference in the shapes of the absorption peaks from the concentrated and dilute solutions will be apparent.

Background Correction

The principal reason for use of the Zeeman effect in atomic-absorption analysis is its facility for accurate correction of the background absorption that arises in electrothermal atomisation. Experiments were carried out to compare its effectiveness and convenience with that of the well established deuterium-arc system. The Glan prism instrument [Fig. 2(*b*)] was designed to permit simultaneous measurement using the deuterium-arc and the Zeeman effect.

As the effectiveness of the deuterium-arc correction system is critically dependent upon coincidence of the optical paths of light from the deuterium arc and the hollow-cathode lamp, particular care was taken in their alignment. Alignment of the Zeeman system was much simpler as only one light source was involved. Trials of the background-correction systems were carried out using the 283.3-nm lead line with a sample matrix of inorganic salts similar in composition to those in blood serum. The correction systems may produce over- or under-correction for background absorption, and consequently an erroneous absorbance signal may be generated, the magnitude of which will be a function of the background absorbance and which is added to the true analytical signal. The variation of this error with background absorbance for samples containing no deliberately introduced lead is shown in Fig. 8.

The extent to which the observed results (Fig. 8) can be attributed to lead contamination can be estimated as follows. If the maximum signal (0.013 absorbance unit) generated in the deuterium-arc system arose from absorption by lead, then there should be a corresponding signal in the Zeeman system of approximately 0.01 absorbance unit. The latter signal is in fact about 0.002 absorbance unit, so that, unless there is substantial malfunction of the Zeeman system, lead absorption cannot contribute more than 20% to the signal generated by the deuterium-arc system. An independent assessment of the lead content of the 10-fold serum equivalent solution gave a concentration of approximately 0.15 mg l^{-1}. The absorbance of this concentration in the Zeeman system would be about 0.002 absorbance unit, hence the deviation of the Zeeman graph is compatible with the known lead contamination. The deuterium-arc measurements were made simultaneously with those of the Zeeman system, and the amount of lead generating a signal was therefore the same for both. When the possible effect of contamination is allowed for in the deuterium-arc measurements, there is still a significant departure from complete background correction. Incomplete background correction arises when the measurement and reference signals are optically or electronically mismatched. The difference in the effectiveness of background correction between the deuterium-arc and Zeeman-effect systems is likely to be optical in origin, as the electronic circuits of both systems were similar. This conclusion is not surprising in view of the difference between the geometries of the two light sources used in the deuterium-arc system and the difficulty in achieving precise optical alignment. These difficulties do not arise in the Zeeman-effect system.

To assess the effect of the background-correction procedures on the measurement of an element against a high background, two sets of serum salt solutions were prepared to which lead was added to give solutions containing 1 and 2 mg l^{-1} of lead. The background-correc-

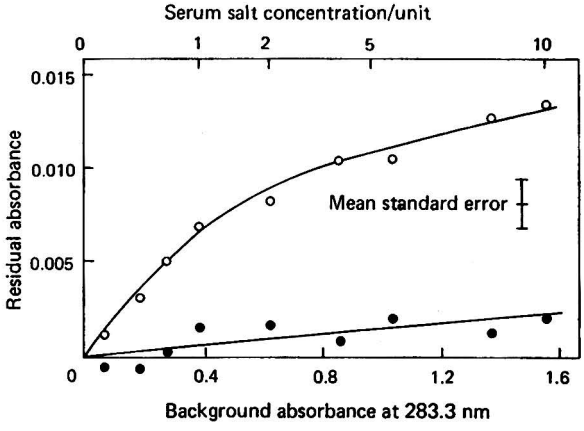

Fig. 8. Dependence of residual error in the signal from Zeeman-effect (8 kG) (●) and deuterium-arc (○) background correction systems on background absorbance generated by serum salts at 283.3 nm. Composition of unit concentration serum salt solution: NaCl, 8.298 g l^{-1}; KCl, 0.328 g l^{-1}; $CaCO_3$, 0.237 g l^{-1}; $MgCl_2.6H_2O$, 0.178 g l^{-1}; $NH_4H_2PO_4$, 0.130 g l^{-1}; H_2SO_4, 0.012 g l^{-1}; HCl to dissolve $CaCO_3$.

ted absorbances of these solutions were measured. At each concentration of matrix salts, the ratio, P, of the corrected absorbance of the 2 mg l^{-1} solution to that of the 1 mg l^{-1} solution was calculated, and the results are presented in Fig. 9. This method of presentation eliminates the effect of the matrix on the atomisation processes of the lead, an effect which caused a 50% reduction in the lead absorbance signal from the most concentrated matrix.

The absorbances attributable to matrix-free lead solutions were 0.026 for the 1 mg l^{-1} and 0.052 for the 2 mg l^{-1} solution, giving an absorbance ratio of 2. If under-correction for background absorption occurs, the absorbance signal contains a contribution from the background and the value of this ratio will fall below 2. The data presented in Fig. 8 can be used to predict the effect of undercorrected background absorption on the measurement of lead. The expression for calculating the ratio P is

$$P = \frac{A_{02} + A_R}{A_{01} + A_R} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (10)$$

where A_{02} is the absorbance of a matrix-free 2 mg l^{-1} lead solution, A_{01} is the absorbance of a matrix-free 1 mg l^{-1} lead solution and A_R is the residual absorbance signal (uncorrected background) for the matrix concentration under consideration. The results of these calculations are shown as the full lines in Fig. 9. There is satisfactory agreement between the calculated curves and the experimental results. Hence, the effect of failure of the background-correction system on the determination of an element can be predicted from measurements made on the matrix alone. However, any effect that the matrix might have on the production of the atomic vapour cannot be overcome by background correction, and it may therefore still be necessary to match the composition of the standards to that of the sample.

A further set of measurements was made using the nickel line at 232.0 nm and a matrix of oil that generated background absorbances of up to 2.0 absorbance units. As the indigenous nickel could not be determined conveniently, a precise estimate of its contribution to the corrected signal could not be made. The correction using the deuterium-arc system was to within 0.035 absorbance unit, while that obtained using the Zeeman system at 7 kG was to within 0.005 absorbance unit.

Using the Zeeman system described in this work, it is possible to detect, with lead, an atomic absorbance of as little as 0.003 absorbance unit against a background absorbance of 1.6 absorbance units. The system has the additional advantage that as the correction takes

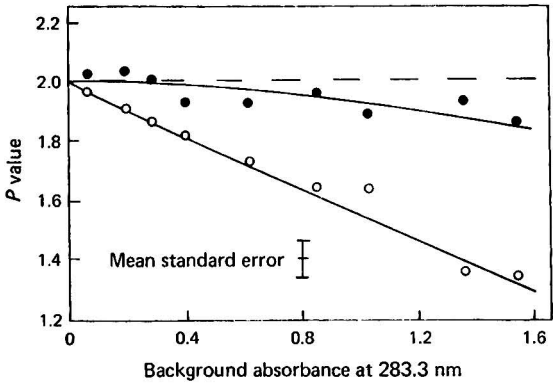

Fig. 9. Dependence of the ratio of the background-corrected absorbances of 2 and 1 mg l^{-1} lead solutions (*P*) on matrix absorbance for Zeeman-effect (8 kG) (●) and deuterium-arc (○) background-correction systems. The full lines are computed curves.

place at exactly the wavelength of the atomic absorption, errors from a rapidly changing or structured background are much less likely to arise. If the background absorbing species exhibit a preferential absorption of one polarisation in the magnetic field, some interferences with the background correction will occur. Such a situation would arise in the event of wavelength coincidence of a background absorption line of either atomic or molecular origin with the analytical absorption line.

Conclusion

These studies have shown that the Zeeman effect generated in the analytical atomic vapour, the inverse Zeeman effect, can be used as an effective method of background correction in electrothermal atomic-absorption spectrophotometry. In common with other methods based on the Zeeman effect, only a single light source is required, which simplifies the optical alignment of the instrument. Further, by utilising polarisation of the light from the lamp, an effective double-beam operation is achieved that corrects precisely for changes in the intensity of the light source. The analytical sensitivity of a Zeeman-effect instrument may be less than that of a conventional background-corrected electrothermal atomisation system because the output signal of the former is the difference between the absorbances of resonance radiation polarised parallel and perpendicular to the direction of the magnetic field applied to the atomic vapour. The absorbance of each component of polarised radiation is the sum of atomic and background absorbances. As the background absorbances of both components are the same, the output signal is determined by the difference between their atomic absorbances. This difference is a function of the magnetic flux density and of the emission and absorption line profiles. At low magnetic flux densities the difference may be small and hence the analytical sensitivity will be reduced compared with that of conventional background-correction systems where only the principal signal, equivalent to the parallel-polarised component of the Zeeman system, undergoes significant atomic absorption.

When tested with samples that generate strong background absorption signals, the Zeeman system compensated background absorbances of 2.0 absorbance units to within 0.005 absorbance unit, which was at least five times better than that obtained with a well adjusted deuterium-arc corrector system used with the same apparatus. In comparison with other methods of background correction, Zeeman-effect systems are most advantageous when the spectrum of the background is structured or when a small signal is to be measured against a large background.

We conclude that the greatest benefit from the use of the Zeeman effect will be obtained in instruments that are designed specifically for its use with electrothermal atomisation.

These benefits will be (1) improved accuracy arising from near perfect background correction, (2) background correction without an additional light source and usable at all wavelengths and (3) a highly stable base-line signal using a simple optical system.

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Determination of Metallic and "Oxide" Copper in Ores

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The selective determination of metallic copper in the presence of copper sulphides and oxides is performed by removal of these oxides or sulphides by means of a cyanide - hydrazine leach solution (pH 12 ± 0.2), washing the residue and dissolving the metallic copper in nitric acid. The copper in this solution is then determined by an atomic-absorption spectrophotometric or spectrophotometric (cuprizone) procedure. The recovery of metallic copper is about 98.6% and the average standard deviation ±0.003% for the range 0.011–0.142%.

The "oxide" copper is dissolved out of another portion of sample by means of an ammonia - ammonium chloride - sodium pyrophosphate leach. The solution is filtered and the copper(I) in the filtrate is oxidised with hydrogen peroxide and the absorbance of the filtrate is measured at 610 nm. The method determines "oxide" copper(I) and copper(II) and neither metallic nor trivalent iron interferes. The average standard deviation is ±0.003% for the range 0.01–1.15% of "oxide" copper.

Keywords: Copper determination; ores; atomic-absorption spectrophotometry; spectrophotometry

Both metallic and "oxide" copper are usual components of oxidised ores and the knowledge of their concentrations and their ratios to the total copper concentration is an important factor in the selection of the most economical process for copper production. All non-metallic and non-sulphide copper compounds [those found in ores are mainly copper(II) oxide, hydroxide, carbonate, silicate and sulphate and their mixed compounds and copper(I) oxide] can be included within the term "oxide" copper.

Many methods have been described for the examination of the combination of copper(I) and copper(II) oxide and metallic copper[1–6] but none for a combination that also includes copper(I) and copper(II) sulphide. Infrared spectrometry[7] and counting with the assistance of a microscope[8] are possible methods, but they are too tedious and inaccurate to be used in routine analysis. The use of sulphuric acid - sulphite[9] and silver sulphate (or nitrate)[6] are the only methods that have been described for "oxide" copper. Both methods are non-selective and determine only about 50% of any copper(I) oxide; in addition, metallic and trivalent iron interfere in the former method.

The use of a cyanide leach[1] for the determination of metallic copper in the presence of copper(I) oxide and an ammonia leach[2] for copper oxides in the presence of metallic copper are known. Both of these methods have been modified to make them selective and universal and the results are described in this paper.

Experimental

Instruments

A Jarrell-Ash, Model 810, atomic-absorption spectrophotometer (Fisher Scientific Co., Waltham, Mass.), a double-beam Cary, Model 118C, spectrophotometer (Varian Associates, Palo Alto, Calif.) with a 1-cm glass flow-cell and a Model 801A pH meter with a 91–01 combined glass electrode (both Orion Corporation, Cambridge, Mass.) were used.

Reagents

Cyanide - hydrazine solution. Dissolve 100 g of potassium cyanide (or 75 g of sodium cyanide) in water, add 50 g of hydrazine hydrate and dilute to 1 l.

Copper(II) - ammonia reagent. Dissolve 50 mg of copper(II) sulphate pentahydrate in 100 ml of 3.5 M aqueous ammonia.

Borate buffer. Dissolve 15.4 g of boric acid, 17.8 g of sodium hydroxide and 18.8 g of potassium fluoride in water and dilute to 1 l.

Cuprizone solution. Dissolve 5 g of cuprizone [bis(cyclohexanone)oxalyldihydrazone (BDH Chemicals Ltd.)] in 1 l of warm (70 °C) 50% *V*/*V* aqueous ethanol.[11]

Metallic copper. Copper powder, 99.5%, passing a 325-mesh sieve (Ventron Corporation, Beverly, Mass.).

Ammonia - ammonium chloride - pyrophosphate reagent. Dissolve 36 g of ammonium chloride and 40 g of sodium pyrophosphate decahydrate in 650 ml of water, add 280 ml of 14 M aqueous ammonia and dilute to 1 l. The solution has pH 10.5 ± 0.2.

Copper(II) standard solution. Dissolve 500 mg of metallic copper in 10 ml of 7 M nitric acid, boil for 5 min to expel nitrogen oxides and dilute to 1 l.

Copper(II) carbonate standard. Determine the copper content of an amount of copper(II) carbonate powder by an electrolytic and/or iodimetric method. The precision of determination should be at least 0.05%.

Procedure for Determination of Metallic Copper

Place an amount of the ore sample (containing 0.1–4.0 mg of metallic copper and having a particle size of less than 150 mesh) into a 100-ml beaker. Add 10 ml of the cyanide - hydrazine reagent and leach with stirring for 2–3 min. Filter using a No. 30 Whatman paper (Note 1) and a short-stem (6–8 cm) funnel to which 6–8 cm of Tygon tubing has been fitted. Wash with at least four 25–30-ml portions of water until the the effluent shows no reaction for cyanide (Note 2). Fasten a screw-clamp on the Tygon tube to seal the funnel stem. Add 10 ml of 7 M nitric acid to the residue on the filter and allow to stand for 15–30 min with occasional gentle circling of the funnel to stir the liquid inside. Introduce the end of the Tygon tube into a 100-ml calibrated flask, remove the screw-clamp to allow the filtrate to run into the flask and wash the residue on the paper with three 25-ml portions of water, collecting the washings in the flask (for an alternative spectrophotometric finish see below). Add 5 ml of 12 M hydrochloric acid, dilute to the mark and mix. Measure the atomic absorption at 327.4 nm employing an acetylene - air flame.[10] Carry out measurements on copper standards containing 1, 5, 10, 20, 30 and 40 μg ml^{-1} and the same concentration of nitric and hydrochloric acid. Calculate the result by using a linear regression programme where y = log [copper] (μg ml^{-1}) and x = log (absorbance) or construct a calibration graph.

NOTES—

1. If another type of filter-paper is used, check its resistance to nitric acid in this apparatus.
2. Mix equal volumes of the effluent and the copper(II) - ammonia reagent. The solution is decolorised in the presence of cyanide.

Alternative spectrophotometric finish

Dilute the solution in the 100-ml calibrated flask to volume with water without addition of hydrochloric acid and mix. Pipette a 5.00-ml aliquot into a 50-ml calibrated flask, add 10 ml of the borate buffer and mix thoroughly in order to decompose the nitrogen oxides that otherwise would destroy the cuprizone reagent. Add 10 ml of cuprizone reagent, dilute to the mark and mix. The pH of this solution must be in the range 8.2–8.6 After 15 min measure the absorbance at 600 nm against a water blank. Measure standard copper solutions containing 20, 50, 100, 150 and 200 μg ml^{-1} in the final solution in the same way. Calculate the result by means of linear regression programme where y = concentration of copper and x = absorbance. After calibration in this way the reading on the spectrophotometer can be directly converted into concentration of copper.

Procedure for Determination of "Oxide" Copper

Place an amount of sample (containing 1–18 mg of "oxide" copper and having a particle size of less than 150 mesh) into a 100-ml beaker. Add exactly 25.00 ml of ammonia - ammonium chloride - pyrophosphate reagent and stir for 10–15 min at room temperature. Filter through a No. 30 Whatman paper and discard the first 5–10 ml of the filtrate. Add 2 drops of 30% hydrogen peroxide to the main portion of the filtrate, mix and after 5 min measure the absorbance at 610 nm against a water blank. Carry out similar measurements on a set of at

least three solid standards covering the expected range of the copper concentration. Calculate the result by means of a linear regression programme where y = concentration of copper and x = absorbance. After calibration in this way, the reading on the spectrophotometer can be directly converted into concentration of copper. If copper(II) carbonate is used as standard, the standard solutions need not be filtered.

Results and Discussion

The results of the determination of metallic copper, including those showing the effect of various factors such as the presence of other substances, the effect of hydrazine and the time of cyanide leach, are presented in Table I. The results of the determination of "oxide" copper showing the effect of metallic and trivalent iron, "oxide" copper(I), copper sulphides and composition of the leaching reagent and the time of leach are collected in Table II.

TABLE I

EFFECT OF VARIOUS FACTORS ON THE DETERMINATION OF METALLIC COPPER

Present				Present			
Amount of metallic Cu/mg	Nature of other substance	Amount of other substance/mg	Amount of metallic Cu found/mg	Amount of metallic Cu/mg	Nature of other substance	Amount of other substance/mg	Amount of metallic Cu found/mg
1.05		—	1.02	3.02	$CuCO_3$	50.12	2.98 (0.5)†
2.56		—	2.57	3.04		51.44	3.02 (1)†
3.01		—	2.97	3.05		49.31	3.01 (2)†
3.98		—	3.95	3.09		50.77	3.04 (3)†
				3.07		50.14	3.02 (5)†
3.10	Cu_2S	10.14	3.02	3.05		52.01	2.93 (10)†
3.06		50.01	3.04	3.06		50.93	2.85 (20)†
3.05		100.33	3.01	3.01		50.73	2.79 (30)†
3.04		150.21	2.99	3.03		100.21	2.95 (50)‡
				3.05		98.33	2.98 (100)‡
2.98	CuS	10.11	1.62*	3.02		99.44	2.99 (200)‡
3.04		50.05	1.19*	3.06		100.35	3.02 (500)‡
3.01		101.21	0.95*	3.01		101.14	2.98 (1 000)‡
3.07		148.95	0.88*	3.03		102.01	2.98 (3 000)‡
3.02		10.50	2.98				
3.03		50.13	2.97	3.06	$CuSO_4$	50.13	3.04
3.09		99.57	3.02	3.02		100.66	2.98
3.11		149.65	3.05	3.03		20.12	2.99
2.99	$CuCO_3$	10.31	2.95	3.01	Cu_2O	51.33	2.97
3.06		50.24	3.03	3.04		111.41	3.01
3.03		101.42	3.00	3.06		150.18	3.02
3.05		151.33	3.03				

* No hydrazine added.
† Time of cyanide - hydrazine leach/min.
‡ Total amount of hydrazine hydrate added/mg.

Typical results of metallic and "oxide" copper determinations in ores are shown in Table III. This table also shows the results of a spectrophotometric determination of metallic copper (all other results including those in Table I were obtained by atomic-absorption spectrophotometry) and the effect of addition of known amounts of the metallic and "oxide" copper.

In a system of metallic and "oxide" copper and copper sulphides, the metallic copper is the most inert component. Therefore, a suitable method for its determination would keep it in its zerovalent state and the cyanide - hydrazine method is that type of method. Reactions (1) and (2) show the ways in which metallic copper can enter the solution.

$$2Cu + 8CN^- + 2H_2O \rightarrow 2Cu(CN)_4^{3-} + H_2 + 2HO^- \quad (1)$$

$$2Cu + O + H_2O \rightarrow 2Cu^+ + 2HO^- \quad (2)$$

The oxidant in reaction (2) can be oxygen from the air or from another reagent. The most important conclusion from (1) and (2) is that both reactions can be suppressed by working

TABLE II

EFFECT OF VARIOUS FACTORS ON THE DETERMINATION OF "OXIDE" COPPER

Amount of Cu in oxide/mg	Substance	Amount of substance/ mg	Amount of $Fe_2(SO_4)_3.9H_2O$/ mg	Average relative error, %	Number of determinations
2.58–13.63 as $CuCO_3$		—	—	−0.34	5
8.91–11.47 as Cu_2O	Cu_2S	100	12–168	1.24	8
7.90–12.34 as $CuCO_3$		100	12–92	1.49	4
9.88–10.56	Cu	11–18	13–105	−1.69	4
10.31–19.04	CuS	84–98	13–520	0.49	6
12.71	Cu_2S	100	100	0.08*	1
10.44		100	100	0.67†	1
11.66		100	100	0.26‡	1
9.73		100	100	1.23§	1
14.78 as Cu_2O	Cu	10	100	−0.41*	1
16.56		10	100	−0.30†	1
10.77		10	100	0.19‡	1
12.21		10	100	0.08§	1
10.71–12.78	Cu_2S	100	100	−48.60‖	4
9.97–10.37 as $CuCO_3$		100	28–164	74.60¶	6
10.06–12.27	Cu	21–59	90–230	53.66¶	2
9.98–10.73		4–17	—	2.60¶	5
9.98–10.63	Fe	13–31	—	−0.88¶	4
5.25–15.05		11–30	100	1.42	4

* 5-min leach.
† 10-min leach.
‡ 20-min leach.
§ 30-min leach.
‖ Without ammonium chloride.
¶ Without sodium pyrophosphate.

in an alkaline medium and by the presence of reducing agents. The fact that the average recovery of metallic copper is 98.6% (see Table I) and the decrease in recovery with the extension of the time (20–30 min) of cyanide leach can be explained by the effect of reactions (1) and (2). Better results are obtained by working in an atmosphere of nitrogen but that is inconvenient for routine analysis.

In accordance with the reactions (1) and (2), Table I also shows that the presence of copper(II) in the absence of hydrazine has a detrimental effect on the determination when reactions (3) and (4) take place.

$$2CuS + 10CN^- \rightarrow 2Cu(CN)_4^{3-} + (CN)_2 + 2S^{2-} \quad .. \quad .. \quad (3)$$

$$2Cu + (CN)_2 + 6CN^- \rightarrow 2Cu(CN)_4^{3-} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

Hydrazine and an alkaline medium (hydrazinium hydrate is used for that reason) suppress reactions (3) and (4) because of reactions (5) and (6).

$$4Cu^{2+} + NH_2NH_2 + 4HO^- \rightarrow 4Cu^+ + N_2 + 4H_2O \quad .. \quad .. \quad (5)$$

$$2(CN)_2 + NH_2NH_2 + 4HO^- \rightarrow 4CN^- + N_2 + 4H_2O \quad .. \quad .. \quad (6)$$

The former reaction has been known for several decades and is used not only for the determination of copper but also for the indirect determination of many other substances.[11]

The effect of the sample matrix must also be taken into account. For example, as reactions (3)–(6) show, copper(II) compounds can consume large amounts of cyanide and hydrazine. The range of metallic copper concentration given in the procedure refers to the usual composition of ores. However, the concentrations of the potassium (or sodium) cyanide and hydrazine hydrate can be increased five times if necessary without any detrimental effect. The concentration of these reagents is sufficient for the quantitative extraction of copper from 100 mg of chalcopyrite.

The spectrophotometric determination of metallic copper is inferior to atomic-absorption spectrophotometry despite the excellence of cuprizone as a copper reagent.[12] The additional

TABLE III

DETERMINATION OF METALLIC AND "OXIDE" COPPER IN ORES

Ore	Metallic Cu found, %	Standard deviation, %*	"Oxide" Cu found, %	Standard deviation, %*
A	0.011	0.001	0.242	0.002
B	0.024	0.001	0.193	0.001
C	0.041	0.002	0.180	0.001
D	0.045	0.002	0.254	0.004
E	0.052	0.003	0.121	0.002
F	0.070	0.004	0.442	0.007
G	0.075	0.004	0.091	0.001
H	0.142	0.007	1.150	0.005
B	0.025	0.001†	—	—
D	0.046	0.002†	—	—
F	0.070	0.003†	—	—
H	0.144	0.006†	—	—
D	0.156 (0.110)‡	—	0.365 (0.113)§	—
E	0.158 (0.155)‡	—	0.346 (0.223)§	—
F	0.154 (0.086)‡	—	0.551 (0.111)§	—

* Result of 5 determinations.
† Cuprizone spectrophotometry.
‡ Amount of metallic copper added, %
§ Amount of "oxide" copper (as $CuCO_3$) added, %

dilution of the solution is required in order to decrease the acidity and the interference of nitrogen oxides, otherwise it would be easy to adjust the amount of sample to meet the sensitivity of the reagent.

The presence of ammonium chloride in the "oxide" copper leach is essential for the determination of the "oxide" copper(I) as the complexes $CuCl_n^{(n-1)-}$, where n lies between 1 and 3, are the only common soluble complexes of copper(I). In the absence of ammonium chloride only about 50% of the "oxide" copper(I) is determined. The oxidation of copper(I) with hydrogen peroxide is necessary as ammonia - copper(I) - chloride complexes exhibit considerably lower absorption of light at 610 nm than ammonia - copper(II) complexes.

Sodium pyrophosphate was found to be a suitable masking reagent for iron(III), which otherwise exhibited a strong oxidation effect on copper sulphides (see Table II) and thus introduced more copper (apparently "oxide" copper) into the solution. The only drawback of this reagent is its relatively low solubility in water. The recommended composition of the leaching solution represents practically a saturated solution of sodium pyrophosphate. The maximum masking capacity of that reagent corresponds to 60 mg of iron(III) or 600 mg of iron(III) sulphate [$Fe(SO_4)_3.9H_2O$]. Pyrocatechol is an even more powerful masking reagent for iron(III) than pyrophosphate with a greater solubility in water. However, it forms coloured complexes with iron(III) so that the determination of "oxide" copper must be performed by atomic-absorption spectrophotometry if pyrocatechol is used. Masking of iron by fluoride or phosphate is inadequate.

As ammonia forms complexes of medium stability with copper(II), the spectrophotometric determination of "oxide" copper has its limit at 18 mg of that element. There is considerable bending of the absorbance - copper(II) concentration graph above that limit. The limit can be increased by increasing the ammonia concentration but solutions with ammonia concentrations higher than 7 M are not stable. The extension of the "oxide" copper leaching time to 30 min has no significant effects on the results.

No specific temperature study of the leaching effect for either method has been performed but the temperature deviations in the range 20–25 °C do not affect the results of the determination.

The substances reported in Tables I and II correspond to actual mineral species. It is well known that the behaviour of some synthetic products such as copper(II) oxide can be very different.

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Ion-exchange Separation and Spectrophotometric Determination of Trace Amounts of Niobium in Silicate Rocks

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A spectrophotometric determination of trace amounts of niobium in rocks and minerals is described. Elemental interferences emanating from the silicate rock matrix are discussed. An ion-exchange separation method is proposed in an attempt to avoid these interferences. A method involving sodium peroxide fusion and hydrochloric acid - hydrogen peroxide leaching is also described. Results for the determination of trace amounts of niobium in international standards of rocks and minerals are shown.

Keywords: *Niobium determination*; *silicate rocks*; *ion-exchange separation*; *spectrophotometry*

The technique for comparing geochemical information has been applied by several workers[1–7] to the classification of lavas from some ophiolite complexes as probable ocean floor rocks, on the basis of their content of trace elements. Niobium, together with zirconium, yttrium, titanium, phosphorus and chromium, is one of the elements that is used to discriminate between fresh tholeiitic and alkaline basalts.

Therefore, the determination of niobium is one of the most important steps in the characterisation of magmatic rocks. Unfortunately, niobium is present in these rocks in ultra-trace amounts (generally less than 10 p.p.m.). In order to determine this low content a colorimetric method is preferred among the techniques commonly available in rock-analysis laboratories because, in atomic-absorption spectrophotometry (the most popular analytical method with rock analysts), niobium is one of the less sensitive elements. The methods used to determine niobium colorimetrically are based on the reactions between niobium and thiocyanate or 4-(2-pyridylazo)resorcinol (PAR). The thiocyanate reaction has been employed by several workers.[8–10] The reaction must be carried out in solution in an organic solvent, preferably after an ether extraction,[11] if the maximum molar absorptivity is to be obtained. The present authors prefer the determination based on the formation of a coloured compound between niobium and PAR, avoiding lengthy manipulation of the sample, which in trace determinations is very deleterious. Alimarin and Han,[12–14] Belcher and co-workers[15,16] and Wood and Jones[17] studied the reaction between niobium(V) and PAR in acetate - tartrate medium at a pH of 5.8 and showed that this reaction is more sensitive than the reaction with thiocyanate.

Experimental

Interferences

The spectrophotometric determination of niobium with PAR is the most selective and sensitive of the various colorimetric procedures. Nevertheless, such a procedure suffers from certain interferences from the elements in the silicate rock matrix. The effects of forty elements on the determination of niobium were studied by Belcher and co-workers.[15,16] In the presence of EDTA (disodium salt), 45 μg of niobium can be determined in the presence of 1 000 μg of lead, copper, bismuth, cadmium, magnesium, iron, aluminium, chromium, silver, zinc, manganese, cobalt, nickel, calcium, strontium, barium, beryllium, tin, arsenic, zirconium, thorium, tantalum, titanium, molybdenum, tungsten and ammonium. Uranium, vanadium and phosphate ions are the only interfering species. The effect of vanadium is eliminated by the addition of metallic zinc, uranium is bound to oxalate, while the effect of tantalum is suppressed by the introduction of excess of tartrate.

Greenland and Campbell[18] at first ignored the selectivity of the reaction and completely separated the niobium in the analysis of rocks. In another procedure[19] they separated niobium from the bulk of the sample and relied on the selectivity of the reaction to prevent interference from the remaining contaminants. In neither of these procedures did these workers draw up a complete list of interfering elements.

Wood and Jones,[17] in their spectrophotometric determination of niobium in zirconium, titanium and other metals, encountered the formation of a zirconium - PAR complex. In subsequent tests the interference from zirconium was overcome by increasing the amount of EDTA used. In fact, a decrease in the concentration of EDTA below the theoretical amount required to react with zirconium caused an increase in absorbance, as unchelated zirconium species reacted with PAR to form a strongly coloured complex. When the concentration of EDTA was increased, the absorbance progressively decreased. This effect was probably caused by increased competition by the EDTA for the niobium ions. Tests performed by Wood and Jones[17] showed that the procedure used in the presence of zirconium was equally applicable to the determination of niobium in the presence of hafnium, tungsten and molybdenum.

When present in an amount in excess of the limiting concentration, copper interferes by forming a coloured complex with EDTA, while iron, nickel and vanadium interfere by forming coloured complexes with PAR and manganese suppresses the development of the niobium - PAR complex. According to Belcher *et al.*[16] and Elinson *et al.*,[20] the interference from tantalum is eliminated by developing the niobium - PAR complex in the presence of a large excess of tartrate at pH 4.5–4.8. Under these conditions, however, the sensitivity of the reaction is considerably reduced and the method is restricted to amounts of niobium above 0.1%.

Meyrowitz[21] stressed that Belcher *et al.*[16] were not concerned with a very high concentration of titanium and that Wood and Jones[17] found it necessary to carry out an extraction of the niobium in order to determine low concentrations of that element in the presence of titanium and iron. In order to overcome the interferences caused by titanium and iron, Meyrowitz[21] established that at pH 6.0 large amounts of aluminium, zirconium, vanadium, chromium, tantalum and fluoride ions did not interfere.

As was described above, iron and titanium are the elements most likely to interfere in the niobium - PAR colorimetric procedure. Uranium, vanadium, zirconium and tantalum interfere when present in large amounts. The chemical means of overcoming these interferences are not very satisfactory; in fact, all additions of EDTA and tartrate salts that are intended to overcome interferences from zirconium, tantalum and other ions are extremely difficult to make effectively and when large they reduce considerably the absorbance, rendering the reaction too insensitive for trace determinations.

Meyrowitz,[21] in order to overcome the interferences from iron and titanium, made no separation of niobium in the analysis of rutile but added the same amounts of iron and titanium present in the portion of the mineral taken for analysis to the standard niobium solutions used to prepare the standard calibration graph in order to compensate for their interference. Nevertheless, such a procedure requires a calibration graph for each sample and strict control of the amounts of iron and titanium added. Several workers[17–19] have found it necessary to extract the niobium in order to determine it.

Principle of the Method

Many workers consider that an ion-exchange procedure for niobium separation is superior to the organic solvent extraction method, at least with regard to the ease of insertion of such a procedure into the analytical scheme. However, the present authors think that a long manipulation of the sample, such as in an organic solvent extraction, is very disadvantageous and that the analytical procedure using an ion-exchange column is more reproducible and precise. Therefore, in our chemical procedures[22–24] we have evaluated a method of separation by ion-exchange chromatography because this procedure is quicker than the extraction procedure and can be used for simultaneous analyses. The ion-exchange separation of niobium has been described by several workers.[25–27] Such procedures involve the use of anion-exchange resins, using hydrofluoric acid solutions as eluents. This technique involves the use of PTFE columns and beakers and great difficulties arise in the manipulation of the hydrofluoric acid solutions. Even though almost complete separation of niobium can be

obtained by use of an anion-exchange procedure, we prefer the use of cationic separation, which is easily incorporated into rock analysis.

The interference from residual interfering ions that pass through the ion-exchange column can be easily overcome. Because niobium is usually present as a complex anion, very little adsorption occurs on a cation-exchange resin.[27] Alimarin and Medvedeva[28] developed a method for the separation of niobium from titanium on KU-2 and SBS cation exchangers in the strongly acidic form from hydrochloric acid solutions containing hydrogen peroxide. Under these conditions titanium forms the cation $[Ti(H_2O_2)_2]^{2+}$ and it is sorbed on the cation exchanger, whereas niobium passes into the eluate in the form of the anion $[NbO_2(O_2)_2]^-$.

In this work the sample is passed through an ion-exchange column filled with Dowex 50-X8 (strongly acidic form) resin. The sample is dissolved in 0.6 M hydrochloric acid containing 0.15% hydrogen peroxide. Niobium completely passes through in the eluate with molybdenum and part of the vanadium present in the rock sample according to their distribution coefficients.[29,30] Such a high molarity of hydrochloric acid was adopted to ensure that all of the niobium present was eluted, even if some interfering elements accompanied it. In fact, Strelow and Victor[31] noted that in perchloric acid niobium is partially retained. The present authors believe, along with Korkisch,[27] that this is a valid point as niobium shows exceptional behaviour in perchloric acid. As was stated in the separation procedure[22] the interfering elements that accompany niobium after sorption of the sample are tantalum, vanadium and molybdenum. All of these elements are present in rock samples as trace elements. However, in the colorimetric determination with PAR, molybdenum and vanadium, which Meyrowitz[21] and Belcher and co-workers[15,16] consider not to be interfering elements at this concentration level, can be easily masked with metallic zinc[15,16] or with EDTA and tartaric acid, added in small amounts so as to depress the absorbance only slightly. In fact, Wood and Jones[17] noted that 50 μg of niobium could be determined in the presence of a 2 000-fold excess of molybdenum with no significant effect on its absorbance; the concentration of molybdenum in common rock samples is of the same order as that of niobium.

Tantalum also reacts to form a coloured complex with PAR. The molar absorptivity of the tantalum - PAR complex is about 8 000 l mol^{-1} cm^{-1}, whereas that of the niobium - PAR complex is about 30 000 l mol^{-1} cm^{-1}, but, because of their relative atomic masses, the absorbance of the tantalum complex is about one eighth of that of the niobium complex. As was reported by Gibalo,[11] the maximum absorbance peak of the tantalum - PAR complex occurs less than 50 nm from that of the niobium - PAR complex; this proximity could well be tolerated by use of a good recording spectrophotometer. In any case, tantalum is present in common rock samples at a concentration about 10 times less than that of niobium.

Dissolution of the Sample

At the trace level, at which it occurs in silicate rock samples, niobium is present principally as a substitute for another major or minor element, *i.e.*, the niobium atoms are not necessarily present as niobate or pentoxide, which are the most common forms of niobium minerals. Niobium may be present as a substitute in spinels and in silicates. For this reason the choice of an appropriate flux depends on the possible form of the niobium. Sodium peroxide was considered by two workers[32,33] to be one of the most powerful fluxes employed in rock dissolution. The choice of alkaline flux depends largely on the subsequent treatment of the melt. A simple leach of the melt with water readily hydrolyses niobates that are decomposed by mineral acids and separates the white, flocculent precipitate of niobium(V) oxide.[11] However, according to Lapitskii and co-workers,[34–36] a compound of the composition Na_5NbO_5 is formed when niobium(V) oxide is fused with excess of sodium hydroxide. This salt is hydrolysed in water with the formation of a 7:6 niobium salt, $Na_{14}Nb_{12}O_{37}.32H_2O$, which is soluble in water.[36,37] Moreover, Gibalo[11] reports that mixtures of hydrogen peroxide with sulphuric or hydrochloric acid are most often employed to form soluble complex compounds with niobium and tantalum. Solutions of niobiotantalum minerals, containing hydrogen peroxide and a mineral acid, are decomposed on boiling, or by treatment with potassium permanganate or when neutralised with ammonia solution. These procedures (accompanied by the separation of the sparingly soluble precipitate of niobic and tantalic acids) have been used by several workers in order to carry out a general separation between the rare earths and the silicate matrix, which includes sodium and nickel from the crucible.[18,38,39]

In this instance, in the separation by ion-exchange chromatography, there exists only the problem of dissolution of the niobium in the sample. The combination of a sodium peroxide flux with a hydrogen peroxide - hydrochloric acid leaching agent appears to be the most powerful dissolution technique considering that (as mentioned above) niobium may not be present with its own compounds but as a substitute for other atoms in a high melting-point mineral such as spinel, which can be fused with ease only by use of the dissolution technique described above.

Method

Reagents

Sodium peroxide.
Hydrochloric acid, 18%.
Hydrochloric acid (6 M) - *hydrogen peroxide* (0.15%) *solution.*
Hydrofluoric acid.
Tartaric acid, 1% *solution.*
Zinc sulphate, 3% *solution.*
Diaminoethanetetraacetic acid, disodium salt, solution, 0.02 M.
Ammonia solution, 6 M.
4-(2-*Pyridylazo*)*resorcinol*, 0.03% *solution.*
Buffer solution. This consisted of 40 g of ammonium acetate and 2.25 ml of glacial acetic acid diluted to 500 ml with water.
Dowex 50W-X8 strongly acidic sulphonate resin, 200–400 mesh.

Apparatus

Eppendorf micropipettes.
Beckman DK-2A recording spectrophotometer. This instrument was equipped with cells of 100 mm path length.

Procedure

Weigh 0.300 0 $\pm$ 0.000 1 g of finely powdered sample into a pure nickel crucible. Add about 1.5 g of sodium peroxide and mix it with the sample powder. Then add a further 1.5-g portion of sodium peroxide in order to form a thin layer of reagent over the sample. Fuse the mixture over a Bunsen burner on an asbestos board to obtain a black liquid melt. Allow the crucible and contents to cool and introduce the crucible (previously cleaned with hot 18% hydrochloric acid) into a large beaker. Carefully add the 0.6 M hydrochloric acid - 0.15% hydrogen peroxide mixture to obtain a clear solution. After washing the crucible with more of the same solution, pass the solution and washings through a borosilicate glass column (22 mm i.d.) filled with ion-exchange resin (Dowex 50-X8, strongly acidic form, 200–400 mesh), loading to a height of 18 cm (about 30 g of dry resin). (The resin column should be pre-treated with 30 ml of the hydrochloric acid - hydrogen peroxide solution.)

Following the passage of the sample solution, wash the column with 50 ml of the acid - peroxide solution. Collect the washings and sample solution in a PTFE beaker, add 5 ml of hydrofluoric acid and evaporate the mixture nearly to dryness. Next dissolve the residue with 5 ml of hot 1% tartaric acid. Let the solution cool and then add 1 ml of 3% zinc sulphate solution. Add 2 ml of 0.02 M EDTA solution and adjust the pH to 6.0 $\pm$ 0.1 with 6 M ammonia solution.

Transfer the solution into a 25-ml calibrated flask with the minimum volume of water (using PTFE beakers, only 1 or 2 ml of water are necessary), then add 10 ml of 0.03% PAR and 0.5 ml of ammonium acetate buffer solution. Dilute to volume, mix well, stand the mixture for 1 h and then measure (against the blank) the absorbance of the mixture in 100-mm cells by using the spectrophotometer, recording from 450 to 600 nm on an expanded scale if necessary.

The niobium contents of the sample solutions are read off from a calibration graph, which is plotted after the preparation of a standard solution of niobium, as indicated by Meyrowitz,[21] whose method requires the gravimetric standardisation of a 200 p.p.m. niobium(V) oxide solution with *N*-phenylbenzohydroxamic acid. In order to plot the calibration graph, pipette appropriate aliquots of this niobium solution and proceed as indicated above.

Results and Conclusion

In this work we have attempted to perform a sufficient, if not complete, separation in order to make the colorimetric determination of trace amounts of niobium easy and rapid. Therefore, we have retained in the method all of the additions necessary in order to complex residual elements that are eluted with niobium. However, these additions have been controlled so as to leave the sensitivity of the method unaltered.

The values given in Table I obtained with the present method are in good agreement with those reproduced from the literature. In the paper by Flanagan[40] there are no data obtained by use of spectrophotometric methods. Because of this agreement, and because the results obtained are accurate, we maintain that these values are of importance in the chemical analysis of the international standards cited in the table.

TABLE I

AVERAGES AND STANDARD DEVIATIONS OF VALUES FOR THE NIOBIUM CONTENT OF SOME INTERNATIONAL STANDARD ROCKS AND MINERALS

Values represent concentration of niobium, p.p.m.

	This work				
Sample*	Average	Standard deviation	Flanagan recommended value[41]	Abbey usable value[42]	de la Roche and Govindaraju value[43]
G-2	11	3	13.5	14	—
GSP-1	28	5	29	29	—
AGV-1 ..	20	6	15	15	—
UB-N	2	1	—	—	1
FK-N	3	1	—	—	—
GS-N	20	3	—	—	—

* G-2 (granite), GSP-1 (granodiorite), and AGV-1 (andesite) are standards distributed by the US Geological Survey. UB-N (serpentine), FK-N (feldspar) and GS-N (granite) are distributed by the Association Nationale de la Recherche Technique (Nancy, France). FK-N and GS-N have no experimental values because they are new standards.

A synthetic sample, containing major and some trace elements, was prepared in order to test the separation technique. Weighed amounts of pure salts of the most important interfering elements were mixed in the appropriate ratios for common rock samples, as was carried out in our previous work.[22] Table II shows the composition of the synthetic sample and that of the basalt BCR-1 (US Geological Survey). Major elements are expressed as oxides; trace elements are expressed as metals.

TABLE II

MATRIX ELEMENT TO NIOBIUM RATIOS OF SYNTHETIC SAMPLE AND BASALT BCR-1

Ratio	Basalt BCR-1	Synthetic sample
SiO_2:Nb	40 370	40 000
Al_2O_3:Nb	10 081	10 000
Fe_2O_3:Nb	9 926	10 000
Na_2O:Nb	2 422	2 500
K_2O:Nb	1 259	1 500
CaO:Nb	5 126	5 000
MgO:Nb	2 563	2 500
TiO_2:Nb	1 629	2 000
Ta:Nb	0.07	0.10
Mo:Nb	0.08	0.10
V:Nb	29.5	30

In order to confirm the reproducibility of the method, the separation was performed on aliquots of the synthetic solution. In six aliquots, each containing niobium at a relative concentration of 0.20 p.p.m., 0.20, 0.24, 0.16, 0.18, 0.19 and 0.20 p.p.m. of niobium were found.

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Spectrophotometric Method for the Determination of Boron in Glasses, Glazes and Ceramic Colours

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A rapid method for the determination of boron in glasses, glazes and ceramic colours is described, which is based on spectrophotometric measurement of the boron - carminic acid complex, and interference effects by other elements present have been investigated. The method has been designed to give maximum accuracy for a spectrophotometric procedure, bearing in mind that up to 20% of boron oxide may be present and that the reagent is very sensitive.

Keywords: *Boron determination*; *glasses*; *glazes*; *ceramics*; *spectrophotometry*

With the advent of X-ray fluorescence spectrometers in the glass and ceramic industries, rapid and accurate results can be obtained for the analysis of complicated colouring materials, glazes and glasses. Some of these analyses would be almost impossible by "wet"-chemical methods and in any event would be so expensive and time consuming as to preclude their use for control analysis.

In the laboratories of the British Ceramic Research Association, glazes and glass ceramics are being analysed in increasing numbers by X-ray fluorescence spectrometry and this in turn means that a large number of boron determinations have to be carried out by "wet"-chemical methods. The method in use prior to the acquisition of an X-ray fluorescence spectrometer was a procedure based on the titration of mannitoboric acid with sodium hydroxide solution.[1,2] This method was time consuming and involved an appreciable amount of manipulation, and, with complex glazes containing lead, zinc, zirconium, etc., the procedure was not always trouble free. It was clear, therefore, that a more rapid procedure was necessary but that an acceptable degree of accuracy should be retained.

A method involving the separation of boron by distillation as methyl borate was first considered.[3] This method required the use of silica apparatus, anhydrous conditions during esterification and evolution of the borate and was time consuming. The separation of boron by pyrohydrolysis[4] at 1 400 °C was tried with some success. The results were satisfactory but only two samples per day could be analysed and the life of the furnace tubes was short. Kochen[5] used an ion-exchange separation followed by determination using a fluoborate ion-selective electrode in a method for use with borosilicate glasses. However, the method produced results with a relative error of −8%, which is not considered to be adequate for the needs of the ceramic industry.

For the spectrophotometric determination of boron it would be desirable to use a reagent that is directly applicable in aqueous solution. Most methods of this type have severe drawbacks. Chromotropic acid[6] is affected by the presence of silica and Victoria Violet[7] is critically dependent on pH. Azomethine H has been used for up to 2% of boron oxide in glass ceramics[8] but requires 4 h for colour development, together with considerable "analyst time."

It was finally decided to attempt to extend a method that had previously been developed for the determination of boron in magnesite refractories, involving the use of carminic acid.[9] The range of content covered was originally 0–0.3% of boron oxide and this paper describes the work carried out in order to extend the range to up to 20% of boron oxide in glazes and glasses.

Experimental

The method in use for the determination of boron in magnesite refractories was briefly as follows. The sample was decomposed by simmering with dilute sulphuric acid and made up to a standard volume, without prior filtration of the insoluble residue. A 4-ml aliquot of the clear supernatant liquid (or of a filtered portion) was then diluted to 25 ml with concentrated sulphuric acid and, after cooling to room temperature, 20 ml of carminic acid reagent in concentrated sulphuric acid were added by pipette. After dilution to 50 ml with concentrated sulphuric acid the flask was allowed to stand for 3 h in a water-bath at 25 °C. The absorbance

was then measured in 10-mm cells at 610 and 730 nm against a control solution prepared by using 4 ml of water in place of the sample aliquot. The reading at 730 nm was made in order to allow a correction to be applied for the titanium content of the sample. Full experimental details of the method and its development have been published previously[9] and it has now been accepted as a British Standard Method.[10]

Changes in Spectrophotometric Procedure and Calibration

Using 10-mm cells, the maximum absorbance that could be tolerated was about 0.45 (4 ml of 30 p.p.m. boron oxide standard solution; colour developed in a 50-ml volume); above this level the calibration was not linear. However, the absorbance scale of the instrument could conveniently be read up to about 0.8 and so 20-mm cells were substituted and the calibration range reduced to 4 ml of 25 p.p.m. boron oxide solution, giving an absorbance of about 0.75. With a sample concentration of 1 g per 100 ml and using a 4-ml aliquot, the range covered was then 0–0.25% of boron oxide. In order to extend the range to 20% of boron oxide in glazes, etc., it was decided to use a 0.5-g sample and dilute to 200 ml initially, followed by a 20-fold dilution.

In the magnesite method, the standard boron solution was prepared from analytical-reagent grade boric acid, which is not a primary standard. It was realised that any slight error in concentration would not be significant when determining small amounts of boron, but at a content of up to 20% of boron oxide this error, if present, could cause problems. Two standard boron solutions were therefore prepared, one from boric acid and the other from freshly re-crystallised sodium tetraborate, which can be regarded as a primary standard. Top calibration standards were then prepared using each of these standard solutions and their absorbances were found to be identical. It appears, therefore, that boric acid can safely be used to prepare the standard boron solution.

Preparation of Sample Solution

An alkaline fusion in a platinum crucible was the method of choice, having been used successfully for the previous volumetric method.[2] It was found that a 0.5-g sample could be decomposed with 5 g of anhydrous sodium carbonate, a fluid melt being obtained over a Meker burner. As is customary with samples that may contain large contents of lead or zinc, the fusion is carried out at as low a temperature and for as short a time as possible so that the risk of attack on the platinum crucible is minimised. The crucible is then placed in a covered beaker and the melt decomposed with dilute sulphuric acid. Insoluble sulphates and silica will be precipitated and zirconium and titanium may be hydrolysed. The resulting solution and residue are transferred into a 200-ml calibrated flask, without filtering, and diluted to the mark. The suspension is then allowed to stand until the supernatant liquid is clear; alternatively, a portion can be filtered through a dry Whatman No. 40 filter-paper into a dry beaker. A 20-fold dilution is then carried out and the boron content determined on a 4-ml aliquot of this solution.

Preliminary results on a sample of borax frit gave duplicate results of 17.23 and 17.28% of boron oxide, compared with accepted titrimetric results of 17.3–17.4%. These results were considered to be very promising and so a study of interference effects by other constituents that may be present in glazes was carried out.

Interferences

For each contaminant, synthetic sample solutions were prepared that contained 0 and 20% of boron oxide plus 20% of the contaminant. The contaminants were added as their soluble sulphates or by fusion of the oxide in sodium carbonate followed by dissolution in sulphuric acid. After dilution, a 4-ml aliquot was taken and the carminic acid complex developed as usual. The absorbances of both solutions were measured at 610 and 730 nm using the zero calibration standard as control. A top calibration standard was also measured.

The following oxide constituents gave no interference effects: Li_2O, Na_2O, K_2O, CaO, MgO, Al_2O_3, Fe_2O_3, Cr_2O_3, CuO, ZnO, CdO, NiO, PbO, Co_3O_4, Mn_3O_4, SnO_2, ZrO_2, CeO_2, Sb_2O_3 and P_2O_5. The large amount of sodium carbonate added as flux also had no effect.

The interference by titanium has been fully investigated previously,[9] when it was found that the presence of 1% of titanium(IV) oxide in the sample gave an apparent boron content of about

0.04% of boron oxide. This effect can be eliminated by measuring the absorbance at 730 nm (due to the titanium - carminic acid complex only) and then correcting the absorbance at 610 nm (due to both titanium - and boron - carminic acid complexes) by the use of a simple equation (see Calculation). It is clear that only the presence of large contents of titanium will materially affect the boron results. Most glazes and glasses contain less than 1% of titanium(IV) oxide, but it is always advisable to check the absorbance at 730 nm. Some ceramic colours and enamels contain up to 20% of titanium(IV) oxide but most of it should be precipitated during the decomposition and dissolution stages.

One constituent that may cause problems is fluoride, of which up to 5% has been found to be present in some enamel colours. It is reported that the addition of aluminium sulphate solution to the leached melt overcomes any interference by fluoride.[11] In order to ascertain the magnitude of fluoride interference, a frit (No. 30) of known boron content was used. Various additions, shown in Table I, were made to the resulting solution and the boron content was determined.

TABLE I

INTERFERENCE BY FLUORIDE AND EFFECTIVENESS OF ADDITION OF ALUMINIUM SULPHATE

Solution	Determined B_2O_3 content, %
Sample No. 30 only	17.29
Sample No. 30 + $Al_2(SO_4)_3$	17.29
Sample No. 30 + 4% F	17.13
Sample No. 30 + 4% F + $Al_2(SO_4)_3$	17.10
Sample No. 30 + 10% F	16.90
Sample No. 30 + 10% F + $Al_2(SO_4)_3$	16.96

It is clear that fluoride causes low results for boron and that aluminium sulphate is ineffective in overcoming this interference. The results for boron oxide are 0.04% low for each 1% of fluoride present.

As a further check, sample No. 30 was fused in the presence of 4% and 10% of fluoride (added as sodium fluoride). It was thought that the fluoride might be preferentially complexed with the silica in the sample. Blank determinations were also carried out, consisting of fusion in the presence of 4% and 10% of fluoride. The results are given in Table II.

From Table II, it can be deduced that the presence of fluoride causes low results for boron owing to the reagent colour being bleached, rather than to a loss of boron by volatilisation or formation of fluoborate. No method of overcoming this effect has yet been found. However, it appears that an approximate correction can be applied by adding 0.04% to the determined boron oxide results for each 1% of fluoride present.

Chloride and nitrate also cause interferences[12] and all work must be carried out using sulphuric acid only.

TABLE II

INTERFERENCE OF FLUORIDE ADDED PRIOR TO FUSION

Sample composition	Determined B_2O_3 content, %
Sample No. 30 only	17.29
Sample No. 30 + 4% F	17.17
Sample No. 30 + 10% F	16.86
Fusion + 4% F	−0.14*
Fusion + 10% F	−0.42*

* The absorbances of these solutions were lower than that of the zero standard solution; the absorption cells were therefore reversed in the instrument in order to obtain an approximate reading.

Results and Discussion

The recommended procedure for the determination of boron in glazes, glasses and ceramic colours is given in the following section.

As a test of the accuracy of the method, six samples of different types and compositions were analysed for boron. The samples are listed in Table III and consist of three standard samples and three laboratory-analysed samples.

TABLE III

COMPOSITION OF SAMPLES

Sample	Boron oxide (B_2O_3), %	Major constituents
Borax frit (B. Ceram. R. A. No. 30)	17.35	SiO_2 51%, Al_2O_3 7%, CaO 13%, Na_2O 8%, K_2O 1%
Glass (USBS No. 93)	12.76	SiO_2 80%, Al_2O_3 2%, Na_2O 4%
Glass (Soc. Glass Technol. No. 2)	12.86	SiO_2 80%, Al_2O_3 2.5%, Na_2O 4%
Glaze (Lab. No. 1)	15.10	SiO_2 45%, Al_2O_3 6%, CaO 3%, Na_2O 7%, PbO 21%
Glaze (Lab. No. 2)	7.42	SiO_2 45%, Al_2O_3 8%, CaO 6%, K_2O 4%, Na_2O 4%, ZnO 1%, PbO 22%
Colour (Lab. No. 3)	9.28	SiO_2 40%, Al_2O_3 17%, Na_2O 6%, K_2O 1%, PbO 16%, Co_3O_4 2%, Cr_2O_3 1%, ZnO 5%

The samples were analysed in quadruplicate, using the 0–20% calibration in each instance. The results are given in Table IV. All of the previously accepted figures were obtained by titrimetric methods.

TABLE IV

RESULTS OBTAINED ON SAMPLES BY PROPOSED METHOD

Sample	Boron oxide (B_2O_3), %		
	Individual results	Mean	"Standard" results
B. Ceram. R. A. No. 30	17.23, 17.28, 17.30, 17.30	17.28	17.35
USBS No. 93	12.56, 12.53, 12.50, 12.50	12.52	12.76
Soc. Glass Technol. No. 2	12.84, 12.76, 12.76, 12.76	12.78	12.86
Lab. No. 1	14.96, 14.96, 14.97, 14.97	14.97	15.10
Lab. No. 2	7.58, 7.58, 7.52, 7.52	7.55	7.42
Lab. No. 3	9.39, 9.41, 9.33, 9.33	9.36	9.28

The results show excellent agreement with the previously accepted figures, the largest error being on USBS No. 93, which was standardised (according to the Certificate) in 1933.

As a test of reproducibility, the B. Ceram. R. A. No. 30 borax frit was analysed ten times for boron. The analyses were carried out as five duplicates, each pair on a separate day, but all by the same analyst. The individual results were 17.23, 17.28, 17.30, 17.30, 17.32, 17.30, 17.38, 17.22, 17.32 and 17.38%, with a mean of 17.30% and a standard deviation of 0.05%.

These results and those in Table IV show that the method is capable of producing results of good accuracy and precision. The method is also free from interference effects by elements that are normally present in the types of samples in question. It is realised that the method is being stretched to the limit of spectrophotometric procedures but, with the application of good analytical techniques and care, it is possible to obtain remarkably good results.

It is possible to analyse about eight samples in duplicate as one batch over a 2-day period, depending on the availability of platinum crucibles. Sample decomposition and dissolution occupies the first day, while dilution and the actual determination of boron is carried out on the second day. As a 3-h colour-development period is required, it is convenient to arrive at the point of addition of the carminic acid at approximately 11 a.m. and then to add the reagent to each pair of solutions at 10-min intervals. This procedure will allow all solutions to be measured after the 3-h period. The spectrophotometer cells should be well rinsed with each solution but should not be washed out with water between samples. The outside faces of the cells should be rinsed with distilled water after filling with sample solution, and polished with a tissue; it is very easy to leave a "bloom" of sulphuric acid on the optical faces.

The method has found wide application in these laboratories and is in fact preferred to the titrimetric mannitoboric acid method previously used.

Recommended Procedure

Reagents

Unless otherwise stated, all reagents should be of analytical-reagent grade, and distilled water should be used throughout the analysis.

Carminic acid solution, 0.5 g l^{-1}. Dissolve 0.1 g of carminic acid in 200 ml of concentrated sulphuric acid, sp. gr. 1.84, with stirring.

Sulphuric acid, sp. gr. 1.84, approximately 36 N.

Sodium carbonate, anhydrous.

Titanium solution, approximately 1 mg ml^{-1} *of titanium(IV) oxide.* To 150 ml of titanium sulphate standard solution (for atomic-absorption spectrophotometry, containing 1 mg ml^{-1} of titanium) add 10 ml of sulphuric acid (1 + 3) and then dilute the solution to 250 ml with water.

Standard Solutions

Boron solution A, 250 p.p.m. *of boron oxide.* Dissolve 0.444 0 g of boric acid in water and dilute the solution to 1 l in a calibrated flask. Store in a polythene bottle.

Boron solution B, 25 p.p.m. *of boron oxide.* Dilute 25 ml of boron solution A to 250 ml in a calibrated flask. Store in a polythene bottle.

Preparation of Sample

The sample for analysis should be ground to pass a 120-mesh British Standard sieve. A non-metallic (*e.g.*, nylon bolting cloth) sieve is preferable.

Decomposition of Sample

Weigh 0.500 g of the prepared sample, previously dried at 110 °C, into a platinum crucible. Add 5 g of anhydrous sodium carbonate and mix intimately with a spatula. Cover the crucible with a lid and heat it over a gas burner, cautiously at first until frothing ceases. Then heat it over a Meker burner at as low a temperature and for as short a time as are necessary to obtain a satisfactory fusion. Allow the crucible to cool and then wash the outside with a small volume of distilled water.

Place the crucible and lid in a 250-ml beaker and add about 50 ml of water. Cover the beaker with a clock-glass and then add slowly 25 ml of sulphuric acid (1 + 3). Dissolution can be aided by the use of a stirring rod but the beaker and contents must not be heated. When decomposition is complete, remove the crucible and lid, transferring the residue to the beaker with a jet of cold water. Scrub the crucible and lid with a rubber "policeman." An insoluble residue, consisting mainly of silica and insoluble sulphates, may remain. Transfer the solution and residue into a 200-ml calibrated flask, without filtering, and dilute to the mark with water.

Determination of Boron

Filter a portion of the solution through a dry 110-mm Whatman No. 40 filter-paper into a dry beaker, discarding the first few millilitres. Do not wash the filter. (Alternatively, the solution can be allowed to settle until the supernatant liquid is clear.) Pipette 25 ml of the clear solution into a 500-ml calibrated flask and dilute to the mark with water. This dilution is suitable for samples that contain up to 20% of boron oxide.

Transfer a 4-ml aliquot of the diluted solution into a dry 50-ml calibrated flask. In a second dry flask prepare a control solution by adding 4 ml of water by pipette. To both flasks add 20 ml of sulphuric acid (sp. gr. 1.84), rapidly with swirling. Quickly replace the stoppers and cool the flasks in running water, then stand them, together with the concentrated sulphuric acid and carminic acid solution, in a water-bath controlled thermostatically at 25 °C.

After about 10 min, add to each flask by pipette 20 ml of the carminic acid solution, allowing an appropriate drainage time. Dilute each solution to 50 ml with sulphuric acid (sp. gr. 1.84), mix and allow to stand for 3 h in a water-bath at 25 °C.

Caution: Use a pipette filler to add the carminic acid solution.

· Measure the absorbance of the sample solution against the control solution in 20-mm cells at 610 and 730 nm.

Calculation

Calculate the true absorbance due to boron at 610 nm as follows:

$$A^{610}_{B_2O_3} = A^{610} - (R \times A^{730}) \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where

$A^{610}_{B_2O_3}$ = true absorbance due to boron complex at 610 nm;
A^{610} = measured absorbance at 610 nm (due to boron and titanium complexes);
A^{730} = measured absorbance at 730 nm (due to titanium complex only);
R = ratio of absorbances of titanium complex at 610 nm and 730 nm, which must be determined for the particular spectrophotometer used (see Correction for Titanium Content of Sample).

Determine the boron oxide content of the sample by reference to the calibration graph.

Calibration

Transfer 0, 1, 2, 3 and 4 ml of the boron solution B into dry 50-ml calibrated flasks and dilute each solution with water to produce a volume of exactly 4 ml. This will give a calibration graph of 0–20% of boron oxide using the sample mass and dilutions given in the procedure. Develop the complex exactly as described above. After 3 h, measure the absorbance of each solution against the zero boron oxide solution in 20-mm cells at 610 nm. From the absorbances prepare a calibration graph, which is rectilinear and passes through the origin. A fresh calibration graph must be prepared for each new supply of solid carminic acid reagent.

Correction for Titanium Content of Sample

The presence of 1% of titanium(IV) oxide in the sample gives an apparent boron oxide content of approximately 0.04%. Some enamels and glazes may contain up to 20% of titanium(IV) oxide but it should be borne in mind that some or all of it may be precipitated during dissolution of the fused melt. The effect of the titanium that remains in solution can be allowed for without determining the amount present.

Transfer by pipette 10 ml of the titanium solution [approximately 1 mg ml^{-1} of titanium(IV) oxide] into a calibrated flask, add 20 ml of dilute sulphuric acid (1 + 3) and dilute to 50 ml with water. Transfer a 4-ml portion of this solution into a dry 50-ml calibrated flask. Into a similar flask pipette 4 ml of water for preparation of the control solution. Develop the complex exactly as for boron. After 3 h, measure the absorbance of the titanium-containing solution against the control solution in 20-mm cells at 610 and 730 nm. Calculate the ratio (R) of absorbances for the titanium complex at these two wavelengths:

$$R = \frac{\text{Absorbance at 610 nm}}{\text{Absorbance at 730 nm}}$$

Substitute this value (approximately 1.75) in equation (1).

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Haematoxylin with Quaternary Ammonium Salts as Spectrophotometric Reagents for Tin(IV)

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Haematoxylin with cetyltrimethylammonium bromide (CTAB) is proposed for the spectrophotometric determination of tin(IV). The addition of CTAB improved the colour reaction of tin(IV) with haematoxylin, resulting in better sensitivity and a longer linear range of the calibration graph. Similar results were obtained with cetyldimethylbenzylammonium chloride aud cetylpyridinium bromide. Oxidised haematoxylin is not a suitable reagent for tin(IV) because of its poor stability in solution.

Keywords: Tin(IV) determination; spectrophotometric reagents; haematoxylin; cetyltrimethylammonium bromide

Haematoxylin has been proposed as a colorimetric reagent for tin(IV).[1,2] However, it has been found that the colour intensity is not proportional to the concentration of tin(IV),[2] and that the pH must be carefully controlled. Recently, the author proposed the use of haematoxylin and cetyltrimethylammonium bromide for the determination of titanium(IV).[3] This paper describes the use of haematoxylin and oxidised haematoxylin in the presence of quaternary ammonium salts for the determination of tin(IV).

Experimental

Reagents

Haematoxylin solutions, 10^{-2} M and 0.1%. Dissolve by warming 3.022 and 1.000 g of haematoxylin, respectively, in 1 l of 95% ethanol.

Oxidised haematoxylin solution, 0.3%. Dissolve 3.00 g of haematoxylin in a solution of 500 ml of water and 200 ml of 95% ethanol, add 20 ml of 5% hydrogen peroxide and heat the solution in boiling water for 15 min. Cool and make the solution up to 1 l with water.

Tin(IV) stock solution, 1000 p.p.m. Dissolve by heating 0.1000 g of tin in 10 ml of concentrated sulphuric acid. Heat until fumes are given off in order to expel sulphur dioxide. Cool and dilute to 100 ml with distilled water.

Cetyltrimethylammonium bromide solution, 10^{-2} M. Dissolve by warming 3.644 g of reagent in 1 l of distilled water.

Cetyldimethylbenzylammonium chloride solution, 10^{-2} M. Dissolve by warming 3.961 g of reagent in 1 l of distilled water.

Cetylpyridinium bromide solution, 10^{-2} M. Dissolve 3.844 g of reagent in 1 l of 20% aqueous methanol.

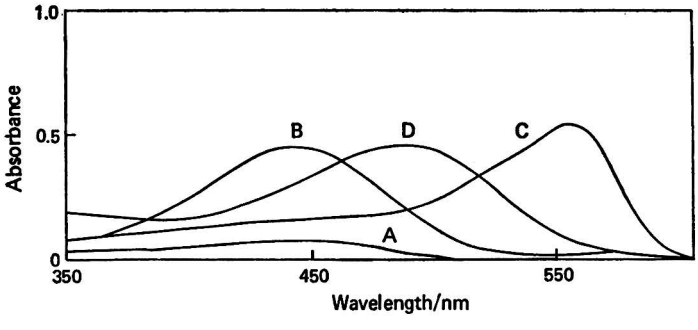

Fig. 1. Absorption spectra of haematoxylin at various pHs: curve A, 0.001% haematoxylin, pH 2.05; curve B, 0.01% haematoxylin, pH 5.20; curve C, 0.001% haematoxylin, pH 7.50; and curve D, 0.001% haematoxylin, pH 9.60.

Development of Method for Tin(IV) and Results

Figs. 1 and 2 show the absorption spectra of haematoxylin and oxidised haematoxylin at various acidities. Fig. 3 shows the effect of cetyltrimethylammonium bromide, cetyldimethylbenzylammonium chloride and cetylpyridinium bromide on the tin(IV) - haematoxylin complex. The effects are similar, the absorption maximum being shifted to about 600 nm.

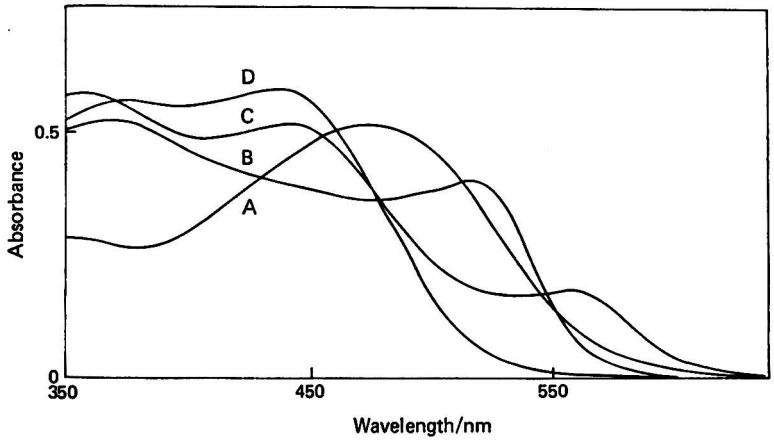

Fig. 2. Absorption spectra of oxidised haematoxylin at various pHs: curve A, 1.25×10^{-4} M, pH 8.80; curve B, 1.25×10^{-3} M, pH 1.15; curve C, 1.25×10^{-3} M, pH 6.05; and curve D, 1.25×10^{-3} M, pH 3.68.

Oxidised haematoxylin and tin(IV) behaved similarly with cetyltrimethylammonium bromide, cetyldimethylbenzylammonium chloride and cetylpyridinium bromide, but with a slight increase in sensitivity (Fig. 4). Haematoxylin was chosen for further study as oxidised haematoxylin was unstable on standing; the reagent became cloudy after a few hours and gave non-reproducible results with tin.

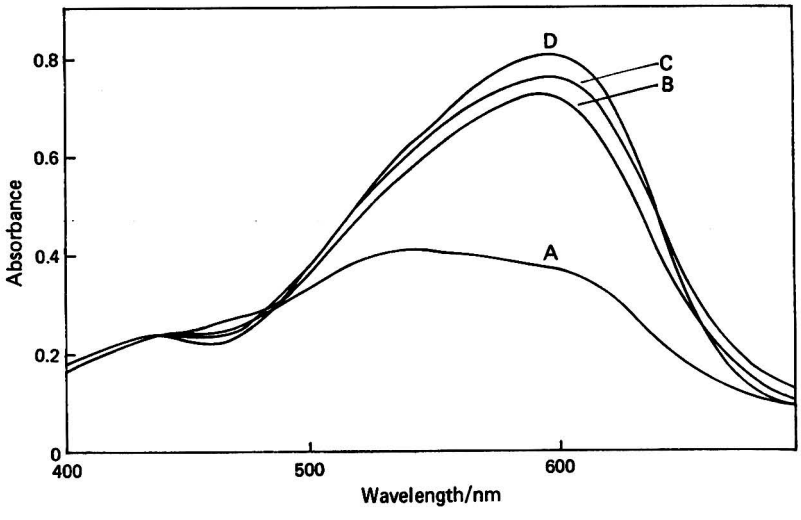

Fig. 3. Effect of quaternary ammonium salts on the tin - haematoxylin complex: curve A, 3.3 p.p.m. tin + 1 ml of 0.1% haematoxylin; curve B, as curve A + 1 ml of 0.1% cetylpyridinium bromide; curve C, as curve A + 1 ml of 0.1% cetyldimethylbenzylammonium chloride; and curve D, as curve A + 1 ml of 0.1% cetyltrimethylammonium bromide; all of the solutions were at pH 1.7.

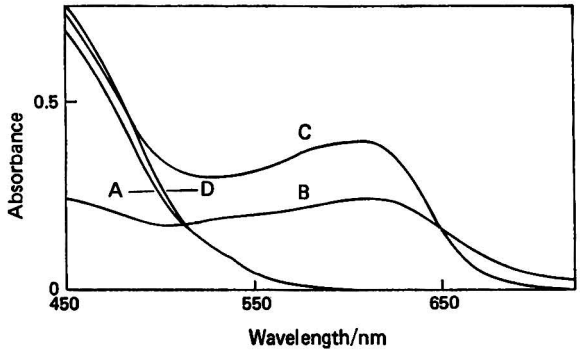

Fig. 4. Effect of cetyltrimethylammonium bromide on the tin(IV) - haematoxylin and tin(IV) - oxidised haematoxylin complexes: curve A, 22.5 ml of 10^{-3} M oxidised haematoxylin in 100 ml, pH 1.7; curve B, 22.5 ml of 10^{-3} M haematoxylin + 10 ml of 10^{-2} M cetyltrimethylammonium bromide + 2 ml of 100 p.p.m. tin solution in 100 ml, pH 1.7; curve C, as curve A + 10 ml of 10^{-3} M cetyltrimethylammonium bromide + 2 ml of 100 p.p.m. tin solution; and curve D, as curve A + 10 ml of 10^{-2} M cetyltrimethylammonium bromide.

Effect of initial acidity and final pH on complex formation

The initial acidity of the tin(IV) solution was found to have an effect on the final absorbance of the tin(IV) - haematoxylin - cetyltrimethylammonium bromide complex. An acidity of 7 N in hydrochloric acid gave maximum absorbance. A study of the effect of pH showed that the solution should have a final pH value of 1.6–1.8 in order to achieve maximum absorbance (Fig. 5).

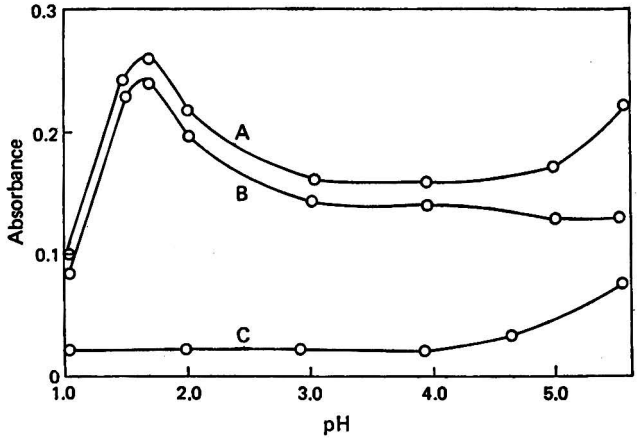

Fig. 5. Effect of pH on the tin(IV) - haematoxylin - cetyltrimethylammonium bromide complex: curve A, 10 ml of 10^{-3} M haematoxylin + 20 ml of 10^{-2} M cetyltrimethylammonium bromide + 2 ml of 100 p.p.m. tin solution diluted to 100 ml and at varying pH, measured against water blank; curve B, as curve A, measured against reagent blank; and curve C, 100 ml of 10^{-3} M haematoxylin + 20 ml of 10^{-2} M cetyltrimethylammonium bromide diluted to 100 ml and at varying pH, measured against water.

Effect of time of development on complex formation

Full colour development of the tin(IV) - haematoxylin - cetyltrimethylammonium bromide complex required about 2½ h; however, 90% of the final absorbance was reached after ½ h.

Stability of haematoxylin solution in 95% ethanol

The age and initial acidity of the haematoxylin solution were found to affect the final absorbance of the complex. Table I summarises the results of a study of these two effects.

TABLE I

EFFECT OF AGE AND INITIAL ACIDITY OF HAEMATOXYLIN SOLUTION ON THE FINAL ABSORBANCE OF TIN(IV) - HAEMATOXYLIN - CETYLTRIMETHYLAMMONIUM BROMIDE COMPLEX

Each solution contained 200 μg of tin.

	Age of haematoxylin solution			
Initial pH of haematoxylin solution	Freshly prepared	24 h	48 h	72 h
3.68	0.320	0.413	0.405	0.395
1.15	0.390	0.386	0.375	0.360
0.95	0.385	0.380	0.361	0.350
0.79	0.362	0.400	0.331	0.330

The following conclusions can be drawn from the results: (a) maximum absorbance of the tin complex was obtained by using haematoxylin solution of initial pH 0.95–1.15; (b) haematoxylin solutions of such acidity were stable for about 24 h, after which they deteriorated; and (c) the use of haematoxylin solutions of pH 0.79 and 3.68 resulted in absorbance values that were initially low. On ageing the reagent for 24 h, the absorbance values obtainable increased substantially, but decreased to lower values after further ageing.

Hence, in order to obtain maximum sensitivity for the colour reaction for tin(IV), freshly prepared haematoxylin solution should be used with its pH adjusted to between 0.95 and 1.15 before use.

Purity of haematoxylin reagent

Supplies of haematoxylin from different sources were found to give solutions with different sensitivities to tin(IV). This effect was traced to the presence of oxidised haematoxylin in some samples. Haematoxylin reagents supplied as stains for microscopic work should not be used as they usually contain some oxidised haematoxylin. Haematoxylin supplied for use as a pH indicator gave satisfactory and reproducible results.

Preparation of calibration graph

Prepare a calibration graph by taking 1.0–7.0 ml of the 1 000 p.p.m. tin(IV) solution, 22.5 ml of 10^{-2} M haematoxylin solution, 10 ml of 10^{-2} M cetyltrimethylammonium bromide solution and 10 ml of 7 M hydrochloric acid. Adjust the pH to 1.7 with dilute hydrochloric acid. Add 5 ml of potassium chloride - hydrochloric acid buffer, pH 1.7, and dilute to 100 ml in calibrated flasks. Measure the absorbance at 590 nm in 10-mm cells against a reagent blank after standing for ½ h.

Sensitivity of reaction

The tin(IV) - haematoxylin - cetyltrimethylammonium bromide system was found to obey Beer's law in the range 0–400 μg of tin in 100 ml of solution, with a molar absorptivity of 2.3×10^4 l mol^{-1} cm^{-1} at 590 nm after a development time of ½ h. Samples should contain 0.1–0.7 mg of tin in the tin(IV) state.

Interference study

The effects of interferences by foreign ions were investigated in solutions containing 200 μg of tin. A foreign ion was considered to interfere when it caused an error in absorbance

corresponding to twice the standard deviation of measurements on the pure tin solution. Cerium(IV), iron(III), aluminium(III), tungsten(VI), bismuth(III), mercury(II), thorium(IV), antimony(III), molybdenum(VI), silver, copper(II), vanadium(V), manganese(II) and nickel-(II) interfered at the 2-mg level. Lead(II), zinc(II) and cadmium(II) at the 10-mg level and gallium(III) at the 5-mg level did not interfere. Common ions including potassium and sodium at the 100-mg level also did not interfere.

Discussion and Conclusion

The presence of cetyltrimethylammonium bromide improved the colour reaction of tin(IV) with haematoxylin. With cetyltrimethylammonium bromide added, the system obeyed Beer's law from 0 to 4.0 p.p.m. of tin(IV), whereas in earlier work[2] with haematoxylin alone it was found that the linear range was from 0.4 to 1.4 p.p.m. The sensitivity was also improved (molar absorptivity with and without cetyltrimethylammonium bromide 2.3×10^4 and 1.7×10^4 l mol^{-1} cm^{-1}, respectively), but there was no improvement in selectivity.

It may be necessary to separate the tin, *e.g.*, by distillation as the halide, if the proposed method is to be used on samples that contain other metallic ions.

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Method for the Separation of Antimony(III) from Antimony(V) Using Polyurethane Foam

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Antimony(III) and antimony(V) in solution can be separated by adjusting the pH to 9.5 and shaking with sodium diethyldithiocarbamate (NaDDC) and polyurethane foam. Alternatively, the solution can be passed through a column of foam that has previously been treated with 5% *m/V* of NaDDC in carbon tetrachloride. The $Sb(DDC)_3$ complex retained by the foam can be eluted with acetone. DDC complexes of iron(III), mercury and silver are also absorbed, but are unlikely to interfere in the subsequent determination of antimony by either atomic-absorption spectrophotometry or neutron-activation analysis.

Keywords: *Antimony(III) - antimony(V) separation; polyurethane foam; water analysis; sodium diethyldithiocarbamate*

In a previous study,[1] we reported a method for concentrating antimony from natural waters, which applied to both antimony(III) and antimony(V). Although it is believed that the toxicity of the element may depend on its oxidation state, there are few techniques that can distinguish antimony(III) from antimony(V).[2–4] Almost nothing is known about the chemical state of antimony in natural waters.

Bode[5] has shown that antimony(III) treated with sodium diethyldithiocarbamate (NaDDC) at pH 7–9 is quantitatively extracted as $Sb(DDC)_3$ in carbon tetrachloride. Wickbold[6] has shown that NaDDC at pH 9 (in the presence of ammonia and tartrate) precipitates the following metals in order of decreasing solubility: iron(III), zinc, antimony(III), cadmium, copper, silver and mercury. DDC does not react with antimony(V), presumably because of the high stability of the oxy anions over a wide pH range and the instability of dithiocarbamate in very acidic solutions. In both instances antimony(V) is separated effectively from antimony(III).

In this work, we have tested the effect of absorbing NaDDC in carbon tetrachloride on to polyurethane foam and the subsequent retention of antimony(III), as well as the absorption of antimony(III) diethyldithiocarbamate on to the foam. The use of polyurethane foam for separation processes was first investigated by Bowen in 1970[7] and since then has been applied by Braun and co-workers[8,9] as a support for stationary phases in extraction chromatography, with many advantages in comparison with other methods, *e.g.*, convenience in handling, recovery and re-use, and the fact that no toxic or flammable solvents are needed.

The process described in this paper separates antimony(III) from antimony(V) and also from arsenic, tin and zinc, but not from silver, cadmium, iron(III) and mercury. The antimony(III) absorbed can be recovered quantitatively by washing with acetone and determined by either neutron-activation analysis or atomic-absorption spectrophotometry.

Experimental

Apparatus

Radioactivity was measured with a well-type sodium iodide detector connected to a Panax counter. Calcium and sodium were determined with an EEL flame photometer.

Reagents

All of the chemicals used, such as sodium diethyldithiocarbamate, antimony metal, metal salts and solvents, were of analytical-reagent grade.

Natural waters (Whiteknights Lake water, Reading sewage effluent and sea water) were filtered through 450-nm Millipore filters within 30 min of collection and stored in acid-washed polyethylene bottles.

A standard 200 p.p.m. antimony(III) carrier solution was prepared by carefully dissolving 0.1000 g of antimony metal in 15 cm^3 of concentrated sulphuric acid and diluting the result-

ing solution to 500 cm^3 with 6 M hydrochloric acid. A standard 200 p.p.m. antimony(V) carrier solution was prepared by carefully dissolving 0.100 0 g of antimony metal in 15 cm^3 of a mixture of concentrated sulphuric acid and concentrated nitric acid (1 + 1) until dissolution was complete, then making up the volume to 500 cm^3 with 6 M hydrochloric acid.

Tracer solutions of antimony-124 in both oxidation states were prepared in the same way as the earlier solutions, using antimony metal that had been activated for 24 h in a thermal neutron flux of about 10^{12} neutrons cm^{-2} s^{-1}. The existence of pentavalent and trivalent antimony in tracer and carrier solutions was confirmed by solvent extraction from 8 M hydrochloric acid with diisopropyl ether.[10]

Silver-110m, arsenic-76, cadmium-109, iron-59, mercury-203, tin-113 and zinc-65 radiotracers in solution were obtained by dissolving known amounts of the metals or oxides in 6 M nitric or hydrochloric acid, following neutron activation at the London University Reactor Centre.

The polyurethane foam used was an open-pore polyether type with a bulk density of 20.9 kg m^{-3}, supplied by Vert Foam Components Ltd., Manchester.

For extraction chromatography, glass chromatography tubes (20 cm long and 2 cm i.d.) were fitted with a separating funnel at the top. Stoppered conical flasks and mechanical shakers were used for batch experiments.

Preparation of Foam and Absorption of Sodium Diethyldithiocarbamate

The polyurethane foam (either cubes of about 5 mm edge or cylindrical plugs of 5 cm length and 3 cm diameter) was washed and dried as described previously.[1] The dried foam was then shaken in a saturated solution of 5% *m*/*V* NaDDC in carbon tetrachloride for 0.5 h to ensure complete saturation. The treated foam was compressed between watch-glasses just before it was used, in order to remove excess of solution.

For column experiments, three treated cylindrical plugs of foam were fitted into each glass tube and they allowed the elution of water samples without any applied pressure.

Determination of Sodium Diethyldithiocarbamate Absorbed on Foam

A column of air-dried NaDDC-treated foam, free from carbon tetrachloride, was transferred into a glass tube and then 50 cm^3 of 2 M nitric acid were eluted through it. The eluate was analysed for sodium using a flame photometer, and the amount of sodium eluted was found to be 3.4 mmol per gram of foam. The amount of NaDDC remaining on the foam was measured after shaking a treated foam with dilute ammonia solution at pH 9.5. It was found to be 0.14 mmol per gram of foam, indicating that most of the NaDDC was loosely absorbed by the foam, while about 4% may have been more strongly absorbed. As the foam is non-ionic, the sodium content was the simplest measure of the NaDDC absorbed.

Results

Batch Experiments

In order to determine the optimum pH range for the separation of antimony(III) from antimony(V) in aqueous solutions, the amount of antimony absorbed on the treated foam was measured at various pH values in batch experiments (Table I). These experiments were carried out in 50-cm^3 water samples (lake water, sea water and treated sewage effluent water; the pH and calcium contents are given in Table II) spiked with radioactive antimony and carrier antimony to give an antimony concentration of 8 μg cm^{-3}. The pH was adjusted with ammonia solution and the samples were shaken with about 0.5 g of treated foam for 0.5 h. For the absorption studies with $Sb(DDC)_3$ we added 0.1 g of NaDDC to the ammoniacal solutions, resulting in a yellow turbidity that clarified after shaking with polyurethane foam for 0.5 h. The amount of antimony(III) retained by the foam in both instances was measured indirectly by comparing the radioactivities of equal aliquots of solution before and after shaking. In all three instances the best separation of antimony(III) from antimony(V) was obtained at pH 9.5.

As $Sb(DDC)_3$ was found to be quantitatively absorbed by the foam, all subsequent work was carried out by adding to the aqueous solution at pH 9.5 sufficient NaDDC to complex all of the antimony to be absorbed in the sample solution. Similar procedures were carried out in order to investigate the optimum shaking time for the retention of $Sb(DDC)_3$ at pH 9.5 and it was

TABLE I

EFFECT OF pH ON THE SEPARATION OF ANTIMONY(III) FROM ANTIMONY(V) FOR FOUR TYPES OF WATER

Batch experiment in presence of excess of NaDDC; 30-min shaking.

Water	pH	Retention on foam, % Antimony(III)	Antimony(V)
Distilled water	7.5	100	0.5
	8.5	100	0
	9.5	100	0
Treated sewage effluent	7.5	80	0
	8.5	84	0.5
	9.5	99	0
Sea water	7.5	96	0
	8.5	99	0
	9.5	98	0
Lake water	7.5	98	0.2
	8.5	100	0
	9.5	99	0

found that absorption of the complex was 80.6% effective after 5 min and 100% after 15 min (mean results for the three types of water sampled).

The extractability of the diethyldithiocarbamate complexes of several radiotracers at pH 9.5 was studied in order to find possible interfering elements with or without the addition of 0.100 g of the disodium salt of EDTA (Table III). Diethyldithiocarbamates of arsenic(III), tin(II) and zinc were not absorbed, while those of silver, cadmium, iron(III), mercury and antimony(III) were largely absorbed by the foam. EDTA completely prevented the uptake of cadmium and iron(III) by treated foam. In practice, all waters containing chloride ions, such as sea water, will interfere with the uptake of silver. The recovery of the metal dithiocarbamates absorbed on to the foam was studied with acetone, ethanol and a 1+1 mixture of ethanol with 2 M nitric acid. The recovery was complete for antimony(III) and silver but only 12.2% of the mercury complex was washed off on shaking with 25 ml of acetone for 15 min.

TABLE II

pH AND CALCIUM DETERMINATIONS IN WATER SAMPLES

Water	pH	Calcium/ $\mu g\ cm^{-3}$
Lake water	8.0	63.5
Treated sewage effluent	7.9	70.5
Sea water	7.8	413

Column Experiments

The elution method was carried out using three cylindrical plugs of foam in each glass tube to obtain a higher absorption capacity for the dithiocarbamate complexes. Such foam columns absorb the antimony - dithiocarbamate complex from all types of water studied (at

TABLE III

RETENTION ON FOAM OF SEVERAL METAL DITHIOCARBAMATES AT pH 9.5

Batch experiment; 15-min shaking.

Metal	Mass of metal/mg per 50 cm^3	Metal retained by foam, %	Metal retained by foam in presence of EDTA, %
$^{110}Ag^m$	5	100	100
^{76}As	0.7	0	0
^{109}Cd	2	82	0
$^{59}Fe(III)$	0.5	100	0
^{203}Hg	0.001	93	86
^{65}Zn	0.5	0	0

antimony concentrations between 0.01 and 40 $\mu g\ cm^{-3}$) at a flow-rate of 5 $cm^3\ min^{-1}$. The foam becomes saturated at antimony concentrations of 130 mg per gram of foam.

The elution of absorbed antimony(III) from the column was achieved with acetone, 25 cm^3 of which eluted 97% of the absorbed antimony at a flow-rate of 1 $cm^3\ min^{-1}$, irrespective of the mass absorbed.

After being dried, the polyurethane foam plugs were ready for re-use.

Discussion

This work forms part of a study of the environmental chemistry of antimony. As both arsenic(III) and arsenic(V) are known to occur in natural waters,[11] similar studies are required for antimony.

Antimony(III) can be separated from antimony(V) by solvent extraction from a variety of acidic solutions,[2–4] but no previous separation from alkaline solutions has been reported. The separation reported here is about as efficient as those using 8 M hydrochloric acid and diethyl ether[2] or ethyl xanthate and carbon tetrachloride,[4] but has a lower distribution constant than that reported by Alian and Haggag[3] for their solvent-extraction scheme. Our method has the advantage of adaptation to column work, in that large volumes of water can be passed down a column that selectively concentrates antimony(III). Liquid - liquid extractions are less suitable for simultaneous extraction and concentration because of the mutual solubility of most pairs of solvents or the need to reduce the volume by evaporation.

Although much of this work was carried out with larger concentrations of antimony than occur naturally, it is expected that very low concentrations would behave in a similar manner. Indeed, the process of absorption on foam is more efficient from very dilute than from more concentrated solutions.[7,12] Unpublished work in this laboratory suggests that antimony in natural waters may be complexed by humic substances, whatever its valency state. However, the dithiocarbamate ion is a much stronger ligand than humic acid for antimony at the pH used here.

Because of the very low concentrations of antimony in most natural waters, the best methods of determining the element appear to be neutron-activation analysis and atomic-absorption spectrophotometry. The former technique uses the 564-keV gamma energy from antimony-122, which is difficult to resolve from the 559-keV peak from arsenic-76 in purely instrumental analysis. The separation technique described above removes most of the interfering arsenic, and gamma rays from silver-110m, mercury-197 or iron-59 are well resolved from the 564-keV peak. Atomic-absorption spectrophotometry requires the generation of antimony hydride for maximum sensitivity, which will prevent interference from any silver or mercury that accompanies the antimony absorbed on to the foam. In flame atomic-absorption spectrophotometry we have improved the sensitivity by a factor of 45 compared with the use of aqueous solutions.

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Vessel for Sampling Liquefied Anhydrous Ammonia for Subsequent Trace Oxygen Determination

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A vessel for the sampling of anhydrous liquid ammonia in pipelines and storage vessels is described. The apparatus allows a representative sample of the liquid ammonia to be taken and completely vaporised. Complete vaporisation of the liquid sample ensures that the gaseous sample subsequently presented to a laboratory gas chromatograph for analysis is truly representative of the original liquid. By using the gas-chromatographic technique described, oxygen can be determined in the range 0.2–50 p.p.m. *V/V*. Hydrogen, nitrogen, argon and carbon monoxide can also be determined on the same chromatogram. Measurements of the oxygen content of ammonia in various storage vessels has allowed an inspection policy for such vessels to be formulated for early detection of stress-corrosion cracking initiated by oxygen. By correct choice of the liquid and expansion volumes of the sample vessel, it could be used for sampling other liquefied gases.

Keywords: *Oxygen determination*; *liquefied anhydrous ammonia*; *sampling vessel*; *gas chromatography*

Over the last 2–3 years there has been heightened interest in the determination of oxygen in the range 1–10 p.p.m. *V/V* in anhydrous liquefied ammonia contained in storage and transport vessels. This is the result of a better understanding of the mechanism of stress-corrosion cracking of steel vessels and of the part played by oxygen in this process. Now that the potential problem has been widely recognised, many companies are pooling information obtained from the examination of vessels and the analysis of ammonia. This information is being correlated in order to produce a tentative code of practice for the examination of storage vessels, based on the probability of stress-corrosion cracking occurring.[1] Results obtained so far suggest that if the oxygen content of the ammonia exceeds about 2.5 p.p.m. *m/m* when the water content is 100 p.p.m. *m/m*, additional precautions, such as an increased frequency of inspection, should be taken.

The most appropriate technique available for the determination of trace amounts of oxygen and other permanent gases in liquid ammonia is a gas-chromatographic method derived from one already in use for the analysis of liquid carbon dioxide. This technique requires the sample to be in the gaseous phase. As with any analysis, the results can only be as good as the original sample, and when dealing with liquefied gases there is always the possibility that a sample present in two phases, gaseous and liquid, will be obtained. Neither phase of this sample would then separately be representative of the original liquid ammonia. Because of the difference between the volatilities of oxygen and other inert gases and that of ammonia, it is necessary to vaporise completely the whole sample, thereby ensuring that the gaseous sample analysed has the same composition as the liquid sample taken from the plant.

This paper describes a sampling device based on the principle of totally vaporising a sample of liquid ammonia. The use of the sample vessel eliminates one of the variables in the sampling and analysis sequence. Future comparisons and correlations of data on the concentrations of permanent gases with corrosion effects will therefore be more meaningful. Such correlations are particularly important in both the long-term study of individual storage vessels and in comparisons of storage installations in different companies or countries.

Experimental

The sample vessel described in this paper allows the complete vaporisation of a true sample of liquid ammonia prior to its introduction into the sample valve of the gas chromatograph that is used for the analysis.

Design and Construction of the Sample Vessel

Several constraints influenced the design and construction of the new sample vessel. Firstly, it had been found that about 1.5 l of sample were necessary in order to purge the 5-ml loop of the chromatograph sample valve and, as it was considered necessary for a minimum of three replicates of each sample to be injected, a minimum of 4.5 l of useable sample at s.t.p. were required. Secondly, the saturated vapour pressure of anhydrous ammonia is about 8 atm at 20 °C and therefore, to avoid condensation, it was deemed advisable that the maximum pressure in the sampling vessel should be 7 atm. Thirdly, the sample vessel had to be absolutely gas-tight for a period of several days, and sufficiently robust to transport samples for several hundred miles from distant manufacturing facilities.

In view of these requirements, a liquid sample volume of 7 ml and a total vapour volume of 830 ml were chosen. These volumes provide approximately 5.6 l of usable sample measured at atmospheric pressure and normal laboratory temperature but stored at a pressure of 6.8 atm. The liquid sample volume and expansion chamber are in a straight line and connected by Hoke ball valves (Fig. 1), thus minimising unswept volumes. An alternative liquid sample volume of 4 ml, giving a final gas pressure of about 4 atm, is available for winter use at lower ambient temperatures.

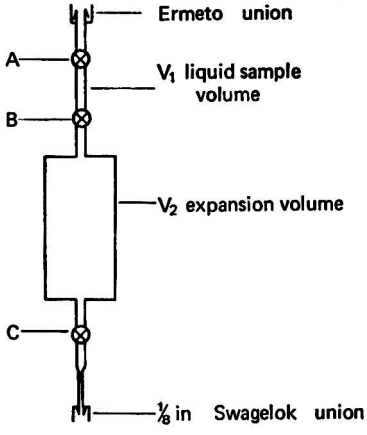

Fig. 1. Vessel for sampling anhydrous ammonia. Valves A, B and C are Hoke ball valves.

When connected to a sample point the vessel must be vertical, with the liquid sample volume uppermost, in order to allow residual liquid remaining in the expansion chamber from the initial purge to be expelled. The sample vessel illustrated in Figs. 1 and 2 is connected to the sample point on the plant or storage vessel with a stainless-steel Ermeto coupling and to the chromatograph by means of a Swagelok union for ⅛ in o.d. tubing.

Chromatographic Analysis

The gas-chromatographic method used for the determination of oxygen and other permanent gases in liquid ammonia was adapted from a method already in use for the analysis of liquid carbon dioxide. The chromatograph was constructed largely from commercially available equipment, arranged as shown in Fig. 2. The helium-ionisation detector and helium carrier gas purification furnace are standard British Oxygen Company models. The detector is similar to that described by Lipsky and Shahin[2,3] and is operated with a polarising voltage of 5 V. The ionisation amplifier used in conjunction with this detector is a Pye Unicam Model 104. The chromatographic column, which is a refinement of that previously described by Hollis[4] and Weems *et al.*,[5] consists of 4 m of ⅛ in o.d. (about 1.5 mm i.d.) stainless-steel tubing packed with 80–100 mesh Porapak Q-S. The column is maintained at a temperature of −78 °C in a Dewar flask packed with solid carbon dioxide and methanol. A column inlet pressure of 15 p.s.i.g. gives a carrier gas flow-rate at the exit of the detector of 2.0 $l\,h^{-1}$. The gas

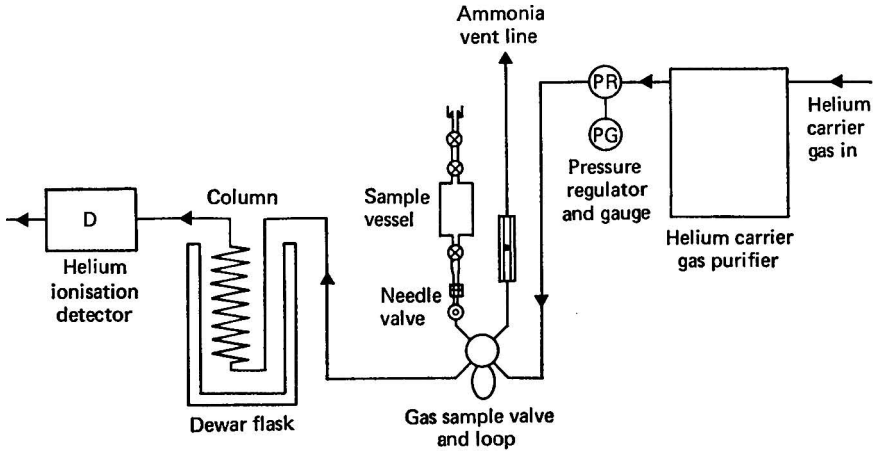

Fig. 2. Sample vessel and chromatograph.

sample valve is a Perkin-Elmer six-port rotary valve, fitted with a 5-ml sample loop. The amplifier output is displayed on a Vitatron recorder with a full-scale deflection of 1 mV and a chart speed of 0.5 cm min^{-1}. A typical chromatogram is shown in Fig. 3.

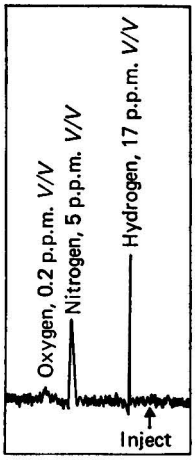

Fig. 3. Chromatogram of Heysham ammonia.

A standard procedure was adopted for purging the sample loop in order to minimise the effects of air diffusing into the carrier gas stream. Both samples and standards were purged through the loop at 1.5 l min^{-1} for 1 min and the loop was then turned into the carrier stream. After a further 20 s the sample valve was returned from the "sample on" to the "purge" position. The chromatograph was calibrated by means of standard gas mixtures produced by an incremental pressure technique. Routine calibration was with a standard cylinder containing each component of interest; the contents of this standard cylinder were themselves analysed by reference to calibration graphs produced from a series of binary mixtures in carbon dioxide. Carbon dioxide was used as it is a condensable gas, like ammonia, but is more convenient to work with and is available in high purity. The calibration graph for oxygen in carbon dioxide is a straight-line graph, passing through the origin.

In normal use about 15 samples can be run over a period of 5 h before break-through of the carbon dioxide or ammonia occurs. The column is then cleared by removing the Dewar flask

and substituting for it a beaker of boiling water for 1 h. In order to avoid the formation of ammonium carbamate, samples of ammonia and carbon dioxide must not be included in the same series.

Sampling Procedure

The use of the type of sample vessel described in this paper required the development of a purging procedure in order to free the vessel from atmospheric oxygen. Previous results had indicated that the ratio of oxygen in the vapour phase to oxygen in the liquid phase might be as high as 1 000:1. Therefore, with true oxygen concentrations in the liquid of about 1 p.p.m. *V/V*, when this sample vessel was purged with liquid ammonia to obtain a good sample of liquid between valves A and B (Fig. 1) the expansion chamber could contain residual ammonia gas with an oxygen concentration of about 1 000 p.p.m. *V/V*. This had to be purged from the expansion chamber until the contribution to the oxygen content of the final vapour sample was negligible (less than 0.05 p.p.m. *V/V* using our chromatograph). The following purging procedure was, therefore, devised (see Note).

NOTE—
Appropriate safety precautions should be taken when sampling anhydrous liquid ammonia. The sample point provided must allow the sample vessel to be positioned vertically, with a minimum 300 mm clearance between the exit from the sample vessel and the floor to prevent splash-back of liquid ammonia.

The sample line is purged and the vessel connected to the sample point. With valves A, B and C (Fig. 1) open, the whole vessel is purged with liquid ammonia for 30 s. Valve B is then shut and the liquid in the expansion chamber allowed to evaporate. When the pressure in the expansion chamber is almost atmospheric, valve C is shut. (When liquid can no longer be seen coming from valve C it is convenient to connect a Drechsel bottle containing about 1 in of oil to the sample vessel outlet so that the point at which the pressure in the expansion chamber is approaching atmospheric can be visually ascertained.)

One purge is next carried out as follows. Valve A is shut, valve B opened and shut, and valve A opened again. The liquid in V_1 thus expands to fill V_2 with vapour. Valve C is then opened until the pressure in V_2 is again nearly atmospheric. Lastly, valve C is shut. This purge procedure is then repeated several times. The final sample is taken in the same manner, except that valves C and A are not opened. The plant sample point is closed and the Ermeto union cautiously broken. Finally, the sample vessel is returned to the laboratory and allowed to attain ambient temperature before its contents are analysed.

Assuming that the initial concentration of oxygen in the residual ammonia gas in the expansion chamber of the sample vessel is about 1 000 p.p.m. *V/V*, and that the pressure of the sample is about 7 atm, then it can be calculated that six purges will reduce this to an insignificant concentration (less than 0.05 p.p.m. *V/V*) compared with the true oxygen content of the sample. A series of three samples was, therefore, taken from the three Billingham ammonia plants' product lines using three, six and nine purges. The results are shown in Table I. It can be seen that six purges should be sufficient for routine use, although nine purges have been used in subsequent work so that a true sample will be obtained even if the oxygen content of the liquid ammonia to be sampled is considerably higher.

TABLE I

SAMPLING OF ANHYDROUS AMMONIA: VARIATION IN CONCENTRATION OF OXYGEN AND ARGON WITH AN INCREASING NUMBER OF PURGES

	Concentration, p.p.m. *V/V*			
Sample	H_2	N_2	O_2	Ar
Expansion vessel, 3 charges purge	283	197	43	75
Expansion vessel, 6 charges purge	103	26	0.7	9.6
Expansion vessel, 9 charges purge	97	25	0.5	8.4

In order to investigate this sampling technique further, results obtained by using this new sampling system were compared with results from samples taken by two other methods. The samples listed in Table II were taken over a period of 2–3 weeks from the Billingham LP ammonia plants' product line. The first method also employed the new sample vessel but

used a vacuum pump to remove residual ammonia vapour from the expansion chamber, V_2, before expanding the liquid sample into V_2 to provide a gaseous sample. Samples taken in this manner are labelled "Expansion vessel, evacuated" in Table II. The second method involved taking a liquid sample in a small pressure vessel and expelling it into a polythene bag in order to allow it to vaporise completely. Because of the traces of air in the empty bag, however, this technique could only be used for the comparison of hydrogen concentrations. Samples taken in this manner are labelled "Vessel expanded into bag" in Table II. It can be seen that the results from all three sampling methods are in good agreement. It appears, however, that with the evacuation technique there is some ingress of air into the expansion chamber while it is below atmospheric pressure.

TABLE II

COMPARISON OF METHODS FOR SAMPLING ANHYDROUS AMMONIA

Sample	Concentration, p.p.m. *V/V*			
	H_2	N_2	O_2	Ar
Expansion vessel, evacuated	140	41	0.7	11
Expansion vessel, 9 charges purge	111	27	0.5	11
Vessel expanded into bag	130	—	—	—
Expansion vessel, evacuated	120	32	1.3	10
Vessel expanded into bag	132	—	—	—
Expansion vessel, 9 charges purge	136	39	0.4	15

Results and Discussion

The development of this sampling technique has permitted the oxygen content of liquid ammonia produced at geographically separated manufacturing facilities to be monitored on a routine basis. Typical results are shown in Table III.

TABLE III

OXYGEN CONTENT OF ICI ANHYDROUS AMMONIA

Sample	Oxygen, p.p.m. *V/V*
Immingham product ammonia	0.5
Heysham product ammonia	0.5
Severnside product ammonia	0.4
Billingham product ammonia	0.5

Most results have indicated oxygen contents of less than 1 p.p.m. *m/m*, and no results have indicated more than 2 p.p.m. *m/m*. Of all the results obtained so far, however, none has shown an oxygen content of less than 0.3 p.p.m. *m/m*. Theoretical considerations of hydrogen - oxygen - water equilibria over the catalysts used in the ammonia process predict oxygen concentrations at least several orders of magnitude lower than the lowest found experimentally. It is probable that true oxygen concentrations are much lower than those found, and that ingress of air at the chromatograph sample valve is giving high blank values of up to approximately 0.4 p.p.m. *V/V* (0.8 p.p.m. *m/m*). Even with this blank the method has proved capable of monitoring oxygen concentrations of less than 2 p.p.m. *m/m*, and there is little incentive for extending the lower limit of the sampling and analytical procedure at present.

Further work on the mechanism of stress-corrosion cracking in a liquid ammonia environment[6] has shown the necessity for both oxygen and nitrogen to be present before stress-corrosion cracking can occur. By using the technique described in this paper, nitrogen concentrations can be determined on the same chromatograph, as also can hydrogen, argon and carbon monoxide concentrations. A regular monitoring procedure for hydrogen, nitrogen, oxygen, argon and carbon monoxide has, therefore, been instituted in our company. The sample vessel described can, by correct choice of the ratio $V_1 : V_2$, be used for sampling other liquefied gases.

Conclusions

A sampling and analytical technique is described, which is capable of monitoring oxygen contents in liquid ammonia of 2 p.p.m. *m/m* and less. Routine use of this technique is pro-

viding valuable evidence on the oxygen concentrations at which stress-corrosion cracking of steel can be avoided. Use of a better gas sample valve may allow lower oxygen concentrations to be determined.

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Determination of Reducing Sugars in Honey, Marmalades and Fruit Juices Using a Copper Ion-selective Electrode

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A potentiometric method for the determination of reducing sugars is described. The sample is treated with Stanley - Benedict reagent and the amount of unreacted copper(II) is determined with a copper-selective electrode by use of the standard addition method; it is then related directly to the amount of reducing sugar. Amounts of glucose in the range 0.25–2 mg have been determined with an average error of about 2%. The method has been applied to the analysis of honey, marmalades and fruit juices. The analytical recovery of glucose was 99–106%. Comparison with an official method gave satisfactory results.

Keywords: *Copper ion-selective electrode*; *standard addition potentiometry*; *reducing sugars determination*; *honey and fruit products analysis*

The official routine methods of analysis to determine reducing sugars in natural products are mostly based on the reducing action of sugars on alkaline copper(II) solution.[1,2] These procedures, involving various techniques, are generally complicated and time consuming.

The use of ion-selective electrodes as sensors for the determination of some reducing sugars, based on reaction rate and continuous flow techniques, has been reported recently,[3–5] but the applicability of these methods has not been tested on natural products.

The present paper describes a potentiometric method for the determination of reducing sugars in natural products, using a solid-state copper(II) ion-selective electrode and a standard addition technique. Following treatment of the sample with a standard amount of Stanley - Benedict reagent, the amount of unreduced copper(II) is monitored with the copper(II) ion-selective electrode. A known small volume of a concentrated copper(II) solution is added and the amount of unreduced copper(II) for each glucose standard or sample calculated by using the standard addition technique.[6] The unreduced copper(II) is directly related to the amount of reducing sugars in the sample under specific conditions and the amount of sugars can be found from a calibration graph prepared with standard glucose solutions. Amounts of glucose in the range 0.25–2 mg were determined with an average error of about 2%. The method was applied successfully to the determination of reducing sugars in honey, marmalades and fruit juices. Recovery and comparison studies carried out on natural samples have shown a recovery of 99–106% and good agreement with official methods.

Experimental

Apparatus

An Orion, Model 94–29 A, solid-state copper(II) ion-selective electrode was used in conjunction with an Orion, Model 90–01, single-junction reference electrode. The potential measurements were made with an Orion, Model 801, digital pH/mV meter. All of the measurements were made in a thermostatically controlled cell at 25 $\pm$ 0.2 °C with continuous stirring by means of a magnetic stirrer. The apparatus was placed in a wooden, black-painted box with a movable front in order to eliminate the response of the copper(II) ion-selective electrode to ambient light fluctuations.[7]

A six-unit, electrically heated micro-Kjeldahl digestion stand (Gallenkamp, Model NR-110) holding six 30-ml Kjeldahl flasks was used for the treatment of the samples with Stanley - Benedict reagent.

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The copper(II) ion-selective electrode was polished with a fine-grain diamond paste (DP-Paste, Type C, H. Struers Scientific Instruments, Copenhagen) and treated with silicone oil every day before use. Between measurements and overnight the electrode was stored dry in air.

Reagents

All solutions were prepared with de-ionised, distilled water from reagent-grade materials, except where stated.

Copper nitrate stock solution, 0.100 0 M. Dissolve 2.416 0 g of $Cu(NO_3)_2.3H_2O$ in water and dilute to 100.0 ml in a calibrated flask. This solution is used for the determination of the slope, S, of the potential *versus* $\log[Cu^{2+}]$ graph and for the standard addition method.

Complexing acetate buffer solution, pH 4.1, 0.2 M. Dissolve 11.8 ml of glacial acetic acid in about 500 ml of water, add 0.84 g of sodium fluoride, adjust the pH to 4.1 with 1 M sodium hydroxide solution and dilute the solution to 1 l.

Glucose standard solutions. Prepare a glucose stock solution by dissolving 10.000 g of glucose in water and diluting to 100.0 ml in a calibrated flask. This solution contains 100.0 mg ml^{-1} of glucose. For the present work puriss.-grade glucose from Fluka was used. Standard glucose solutions containing 0.250, 1.00 and 2.00 mg ml^{-1} are prepared from the stock solution by dilution.

Stanley - Benedict reagent, 0.1 M in copper(II) sulphate and 1 M in sodium carbonate. Dissolve 25 g of copper(II) sulphate (iron-free) in 100 ml of water, 50 g of citric acid ($C_6H_8O_7.H_2O$) in 50 ml of water and 388 g of sodium carbonate decahydrate (or a corresponding amount of the anhydrous salt) in 300–400 ml of warm water. Pour the citric acid solution into the carbonate solution and then add the copper sulphate solution. Dilute with water to 1 l. After the solution has been allowed to stand for several days, filter or siphon off the supernatant liquid from the deposit.[2]

Procedure

Determination of the slope S *of the graph*

The slope is determined from a calibration graph of E, mV, *versus* log (copper concentration), prepared from a series of portions of standard 0.100 0 M copper solution (from 5 μl to 0.5 ml) in a solution consisting of 25.0 ml of complexing buffer and 25.0 ml of water. The slope should be checked once every day before the sample measurements are made.

Sample treatment with Stanley - Benedict reagent

Transfer 1.00 ml of glucose standard or unknown sample solution into a 30-ml Kjeldahl flask, add 0.50 ml of Stanley - Benedict reagent and 8.50 ml of water. Add some anti-bumping granules to avoid excessive foaming and boil the solution on an electric heater for 10 min. Cool the solution to room temperature and filter it through a Whatman No. 42 filter-paper into a 100-ml calibrated flask. Wash the Kjeldahl flask and the copper(I) oxide precipitate on the filter twice with 10 ml of water into the calibrated flask, add 50.0 ml of complexing buffer to the flask and dilute to the mark with water (solution A).

Standard addition procedure

Pipette 50.00 ml of solution A into the cell, thermostatically controlled at 25.0 °C, immerse the electrodes, allow the potential to equilibrate for about 1–3 min and then measure the potential. Add 0.100 ml of the 0.100 0 M standard copper solution with a 0.1-ml Hamilton microlitre syringe and again measure the potential.

Preparation of the calibration graph

Find the unknown total concentration, C_0, of the unreduced copper(II) for each glucose standard from the observed potential change, ΔE, following the standard addition, by using the equation[6]

$$C_0 = \frac{C_\Delta}{\text{antilog } \Delta E/S - 1} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where C_Δ is the change in copper concentration after the addition of the known solution.

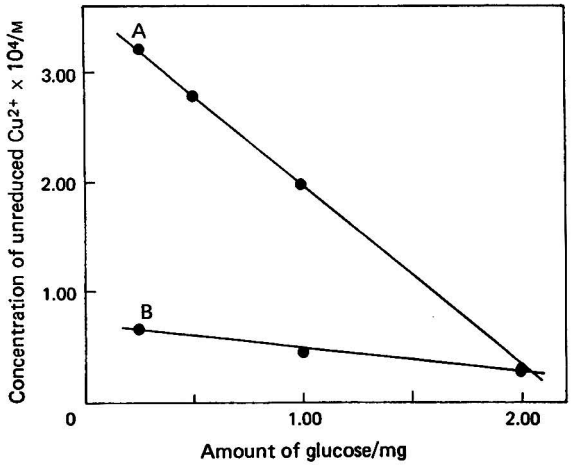

Fig. 1. Calibration graphs for the determination of reducing sugars. Effect of the volume of Stanley - Benedict reagent: A, 0.50 ml; B, 0.25 ml.

Prepare a calibration graph by plotting C_0 against milligrams of glucose. A typical calibration graph is given in Fig. 1 (graph A).

Determination of reducing sugars

Marmalades must be pulped by using a blender at a low speed, whereas honey and concentrated fruit juices must be homogenised by stirring or shaking before portions are weighed out for the sample preparation. Prepare samples by weighing accurately 0.1–0.2 g for honey, 0.2–0.4 g for marmalades and 1–2 g for fruit juices. Dissolve each sample with water in a 100-ml calibrated flask and dilute to the mark (solution B). Proceed as described for the glucose standards, using 1.00 ml of solution B, and calculate the amount of reducing sugars from the calibration graph.

Results and Discussion

The performance of the Orion copper(II) ion-selective electrode is affected by the condition of the electrode surface. The slope and stability are much lower, and the response time longer, when the surface is pitted. Polishing the surface with diamond paste and treatment

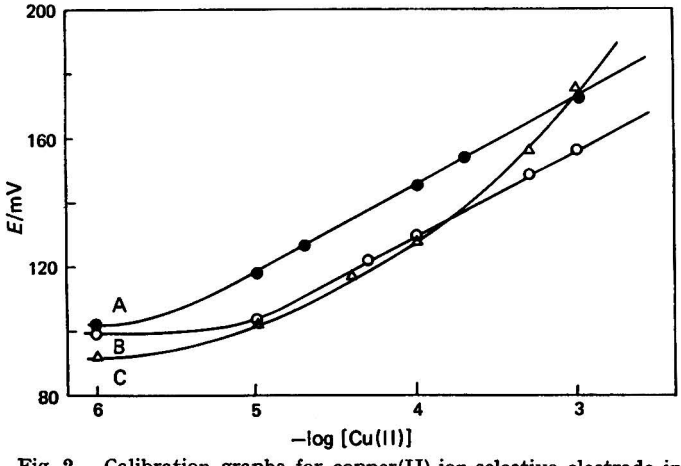

Fig. 2. Calibration graphs for copper(II) ion-selective electrode in copper(II) solutions. A, Aqueous copper(II) solutions; B, with 50 g l^{-1} of citric acid; C, with 346 g l^{-1} of Rochelle salt.

with silicone oil improves the electrode significantly.[8] Isolation of the electrode from fluctuations of the ambient light during measurement further improves its stability.

The choice of the Stanley - Benedict reagent instead of Fehling's solution was based on the following reasons. First, from a study of the electrode behavior, calibration graphs in buffered (pH 4.1) copper solutions (Fig. 2) showed that the Rochelle salt of Fehling's solution seriously affects the linearity of the calibration graph (graph C), whereas in the presence of citrates only a parallel shift of the calibration graph is observed (graphs A and B). Secondly, the Stanley - Benedict reagent has a considerable advantage over Fehling's solution in that the warming of the solution plays a secondary role. Glucose and fructose give the same reduction values for a boiling time of 10 min.[2]

The volume of the Stanley - Benedict reagent used affects the sensitivity and accuracy of the method (Fig. 1). Thus, for glucose in the range 0.25–2 mg, the concentration range of the unreacted copper(II) was reduced from 3.2×10^{-4}–3.0×10^{-5} M with 0.5 ml of Stanley - Benedict reagent (graph A) to 6.6×10^{-5}–2.5×10^{-5} M with 0.25 ml of reagent (graph B). Therefore, a volume of 0.5 ml of reagent was chosen for the recommended procedure. Larger volumes of Stanley - Benedict reagent decrease the sensitivity of the standard addition method.

A boiling time of 10 min was chosen, because with longer boiling times a considerable evaporation of the samples was observed. Shorter boiling times resulted in the formation of a colloidal suspension of copper(I) oxide.

TABLE I

RESULTS FOR AQUEOUS GLUCOSE SOLUTIONS

Glucose/mg			
Taken	Found*	ΔE/mV	Relative error, %
0.250	0.250, 0.250	5.4, 5.4	0.0, 0.0
0.500	0.500, 0.500	6.0, 6.0	0.0, 0.0
1.00	0.96, 1.03	7.6, 7.9	−4.0, +3.0
1.50	1.51, 1.47	11.1, 10.9	+0.7, −2.0
2.00	2.07, 2.09	27.1, 27.5	+3.5, +4.5
			Average: 1.77

* From calibration graph.

TABLE II

COMPARISON OF POTENTIOMETRIC AND AOAC METHODS FOR THE DETERMINATION OF REDUCING SUGARS IN HONEY, MARMALADES AND FRUIT JUICES

Sample	Potentiometric method			AOAC method: amount of reducing sugars found*/ g per 100 g of sample	Difference, %
	Amount of sample taken/g	Amount of reducing sugars found* Actual/g	Amount of reducing sugars found* g per 100 g of sample		
Honey (brand I)	0.123 9	0.084 2	68.0	69.2	−1.7
Honey (brand II)	0.146 2	0.094 2	64.4	64.3	+0.2
Honey (brand III)	0.191 5	0.118 3	61.8	60.2	+2.7
Honey (brand IV)	0.172 9	0.104 2	60.3	61.4	−1.8
Strawberry marmalade	0.389 7	0.197 8	50.8	47.5	+6.9
Orange marmalade	0.321 1	0.114 9	35.8	34.5	+3.8
Peach marmalade	0.292 9	0.053 8	18.4	19.6	−6.1
Cherry marmalade	0.204 8	0.054 5	26.6	25.6	+3.9
Orange juice (canned)	1.752 0	0.147 6	8.42	8.62	−2.3
Grapefruit juice (canned)	1.093 0	0.073 8	6.75	6.72	+0.4
Lemon juice (concentrated)	1.938 2	0.030 9	1.59	1.58	+0.6
Tangerine juice (concentrated)	1.093 0	0.135 7	12.4	12.9	−3.9

* Average of two determinations for each sample and method.

A sodium acetate - acetic acid complexing buffer (pH 4.1) was chosen for the determination of the unreduced copper(II) concentration. This buffer not only adjusts the pH but also serves to de-complex copper from all but very strong complexing agents.[9]

Results for the determination of aqueous solutions of glucose are given in Table I. It can be seen that amounts of glucose in the range 0.25–2 mg can be determined with an average error of about 2%. The coefficient of variation for six replicate complete analyses of 1.00 mg of glucose was 2.7%. The proportionality between the concentration of unreduced copper(II) and glucose is linear (Fig. 1, graph A) in the 0.25–2 mg of glucose range. The relationship was found to be non-linear at lower or higher glucose concentrations. Therefore, the reducing sugars concentration of the samples, expressed in terms of glucose, should be brought into this range.

The method was applied to the determination of different brands of honey, marmalades and fruit juices commonly found on the Greek food market. Several samples were determined by both the proposed method and the official AOAC method.[1] Typical results are shown in Table II. The results are also shown in a correlation graph (Fig. 3). There is satisfactory agreement between the results obtained by use of the two methods. The slightly poorer agreement for the marmalade samples may be a result of the presence of preservatives and the differences in the preparation of the samples.

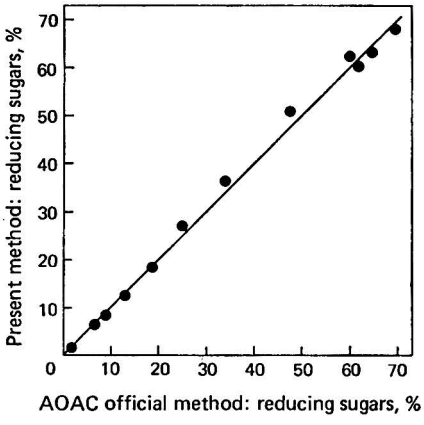

Fig. 3. Comparison of the potentiometric and official (AOAC) methods for the determination of reducing sugars in honey, marmalades and fruit juices. Correlation coefficient, 0.998; slope, 1.008; intercept, 0.023.

The accuracy of the proposed method was further checked by means of recovery experiments carried out on representative samples of honey, marmalade and fruit juice, in which glucose was added to the sample. The results are shown in Table III.

TABLE III

RECOVERY OF GLUCOSE ADDED TO HONEY, MARMALADES AND FRUIT JUICES

Sample	Amount of sample taken/g	Amount of reducing sugars found in sample/g*	Amount of glucose added/g	Total reducing sugars found/g*	Recovery, %
Honey (brand IV)	0.172 9	0.104 2	0.050 0	0.157 1	105.8
Cherry marmalade	0.204 8	0.054 5	0.050 0	0.104 2	99.4
Orange juice	1.752 0	0.147 6	0.050 0	0.199 4	103.6
				Average:	102.9

* Average of two determinations.

The reproducibility of the method was checked by multiple analysis of various samples, as is shown in Table IV. It can be seen that the proposed method shows a high degree of reproducibility, with coefficients of variation ranging from 0.6 to 3.7% ($n = 6$).

TABLE IV

REPRODUCIBILITY OF REDUCING SUGARS DETERMINATION BY THE POTENTIOMETRIC METHOD IN HONEY, MARMALADES AND FRUIT JUICES

Sample	Number of determinations	Mean value/g per 100 g	Standard deviation	Coefficient of variation, %
Honey (brand IV)	6	60.3	0.63	1.0
Cherry marmalade	6	26.6	0.98	3.7
Orange juice	6	8.42	0.05	0.6

In conclusion, the proposed method provides a satisfactory potentiometric alternative for the determination of reducing sugars. The use of the copper(II) ion-selective electrode, which reduces the time of analysis and simplifies the procedure, offers competitive advantages over the conventional manual methods.

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Determination of Residues of Organophosphorus Pesticides in Fruits and Vegetables

Report by the Panel on Determination of Residues of Certain Organophosphorus Pesticides in Fruits and Vegetables

OF THE COMMITTEE FOR ANALYTICAL METHODS FOR RESIDUES OF PESTICIDES AND VETERINARY PRODUCTS IN FOODSTUFFS OF THE MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

Keywords: *Organophosphorus pesticide residues*; *fruits*; *vegetables*; *gas - liquid chromatography*

The Panel was set up by the Committee for Analytical Methods for Residues of Pesticides and Veterinary Products in Foodstuffs of the Ministry of Agriculture, Fisheries and Food in 1973 and first met in February of that year. The twelve laboratories that took part in the work and people representing them are listed in Appendix IV.

Aim

The aim was to investigate and, where appropriate, recommend suitable multi-residue gas - liquid chromatographic methods for a wide range of organophosphorus pesticide residues in as wide a range of fruits and vegetables as was feasible. It was recognised at the outset that not all crop - pesticide combinations which appear in the FAO/WHO Codex Alimentarius Commission proposals for maximum residue limits or in the (then proposed) EEC directive on pesticide residues in and on fruit and vegetables could be examined. The pesticides the Panel studied are representative of several types of chemical structure found in this class of pesticides and are of undoubted commercial importance. The collaborative exercises were designed to cover the range of pesticides, as far as was possible, in seven kinds of fruit and root or leafy vegetables.

Samples for Analysis

Collaborating laboratories provided their own samples of fruit and vegetables that had been screened to ensure that they contained no measurable amounts of pesticide residues. Authentic samples of each pesticide (>95% pure) were provided for spiking purposes from one source. Each laboratory prepared its own standard solutions in organic solvents from these samples. In each recovery experiment of any one series, spiking was carried out by adding the same amount of diluted pesticide solution to each replicate portion of the substrate in the macerator jar and homogenising well with extraction solvent. The levels for recovery experiments were generally those of the suggested Codex or EEC maximum residue limits; these levels were chosen as being the most practical as such determinations would be the most critical. It was recognised, however, that had resources permitted, data at both lower and higher levels would have been valuable. Blank values were subtracted before reporting recovery data in this work.

Criteria for Acceptability of Results for Methods

Criteria for acceptability of results for methods have been adopted. Results for any collaborative study of a crop - pesticide combination should be such that recovery values normally lie between 70 and 110% and ideally the mean should be between 80 and 100%. A few results below 70% and above 110% are also acceptable provided that, when tested by the method of Youden[1] (Dixon's test[2]), they do not prove to be outliers.

Blank values should generally be less than one tenth of the maximum residue limit although it was recognised that in some instances where the level is low, *e.g.*, omethoate, this may not be achieved.

Introductory Work

Following an assessment in one laboratory of five possible methods the panel might consider, those of Abbott *et al.*[3] and of Sissons and Telling[4] were selected for immediate study. Initial collaborative studies indicated that techniques for evaporation of solvent in both methods should be described more clearly and that there were problems concerning column chromatography by the Sissons and Telling method that needed to be resolved. At this stage, the modified Watts *et al.* method[5] of the National Food Institute in Denmark was also investigated and was considered to be promising. The Panel decided to study the method of Abbott *et al.* in depth first, and then to proceed to the modified Watts *et al.* method. A revision of the Sissons and Telling method was examined in detail later.

Abbott *et al.* Method[3]

A detailed revision of the method of Abbott *et al.* (Appendix I) was prepared in which a small amount of propylene glycol "keeper" remained after evaporation in order to avoid the extract being taken completely to dryness. This revised method was examined by collaborative study for recovery of dichlorvos, dimethoate, azinphos-methyl and parathion from carrots, runner beans and tomatoes. The results for dichlorvos, dimethoate and parathion are given in Table I and were satisfactory. Blanks were also acceptable (<0.03 mg kg^{-1}). The validation of the method was extended in another round of collaborative study, recovering these pesticides together with malathion and omethoate from carrots, apples and peas, and results for dichlorvos, dimethoate and malathion are given in Table II. These results were acceptable, as also were the blanks. In a further round of study, dichlorvos, dimethoate and malathion were recovered from lettuces, as a typical chlorophyll-containing leafy crop, and the results are given in Table III.

TABLE I

RECOVERY OF DICHLORVOS, DIMETHOATE AND PARATHION FROM CARROTS, RUNNER BEANS AND TOMATOES BY THE ABBOTT *et al.* METHOD (APPENDIX I)

		Recovery, %		
Crop	Laboratory	Dichlorvos (0.5 mg kg^{-1})	Dimethoate (2 mg kg^{-1})	Parathion (0.7 mg kg^{-1})
Carrots ..	1	92, 97	77, 69, 90, 92	65, 65, 70, 68
	2	93, 95	99, 94	92, 86
	3	79, 82	84, 77	71, 75
	4	71, 78	89, 92	81, 86
	Mean	86	86	76
	Standard deviation	±10	±9	±10
Runner beans	2	94, 91	97, 101	91, 94
	5	62, 65, 73, 75	86, 94, 98, 105, 106	85, 87, 90, 92, 93
	6	96, 99	88, 91	74, 80
	7	91, 89	90, 95	96, 93
	8	78, 86	68, 70	69, 56
	Mean	83	91	85
	Standard deviation	±12	±12	±12
Tomatoes ..	2	92, 96	103, 98, 96	97, 87, 86
	9	—	—	93, 98, 90, 90
	10	98, 96, 102, 97, 93, 99	94, 96, 99, 88, 85, 103	93, 95, 97, 91, 88, 96
	Mean	97	96	92
	Standard deviation	±3	±6	±4
All three crops	Mean	88	91	85
	Standard deviation	±11	±10	±11

In the second of these studies, the recovery of omethoate was low and complicated by uncertainties as to which gas - liquid chromatographic peak should be measured. In applying the method generally to residues of the more polar type of organophosphorus pesticides and

TABLE II

RECOVERY OF DICHLORVOS, DIMETHOATE AND MALATHION FROM CARROTS, APPLES AND PEAS BY THE ABBOTT *et al.* METHOD (APPENDIX I)

Crop	Laboratory	Recovery, % Dichlorvos (0.5 mg kg^{-1})	Dimethoate (2 mg kg^{-1})	Malathion (2 mg kg^{-1})
Carrots ..	2	84, 91	98, 94	92, 95
	5	81, 75	88, 85	88, 79
	6	95, 89	100, 98	97, 99
	11	89, 90	100, 100	93, 98
	Mean	87	95	93
	Standard deviation	±6	±6	±7
Apples ..	7	71, 87	94, 100	102, 107
	8	111, 114	89, 93	98, 100
	10	88, 94, 91	96, 101, 106	95, 101, 105
	Mean	94	96	101
	Standard deviation	±15	±8	±4
Peas	1	73, 99	70, 71, 91, 78	93, 95
	4	89, 95	83, 87	87, 88
	5	94, 96, 104	92, 90	89, 87
	9	86, 82	84, 87	92, 89
	Mean	91	83	90
	Standard deviation	±9	±8	±3
All three crops	Mean	90	91	94
	Standard deviation	±10	±9	±7

their metabolites and to polar metabolites of less polar pesticides, it has been found that many of these compounds are not favourably partitioned between an aqueous phase and an organic solvent in the early clean-up stages. In such instances a slight modification of the studied method (as indicated in Appendix I) was necessary in order to obtain sufficiently favourable partition characteristics. The recovery of omethoate from apples, carrots, lettuces and plums is given in Table IV. When the unmodified Abbott *et al.* method is used on an "unknown" sample, the more polar type of organophosphorus pesticide will appear on the chromatogram if it is present in significant amounts, but the extraction efficiency and hence over-all recovery may be less than 50%. If a quantitative assay is then required the modification must be used on a fresh extract.

Further investigation of the modified method for such compounds as oxydemeton-methyl (demeton-*S*-methyl sulphoxide) and demeton-*S*-methyl sulphone has been hampered by

TABLE III

RECOVERY OF DICHLORVOS, DIMETHOATE AND MALATHION FROM LETTUCES BY THE ABBOTT *et al.* METHOD (APPENDIX I)

Laboratory	Recovery, % Dichlorvos (1 mg kg^{-1})	Dimethoate (2 mg kg^{-1})	Malathion (2 mg kg^{-1})
1	74, 76	93, 51*	74, 76
2	88, 86	98, 99	97, 97
5	82, 89, 84	98, 100, 106	95, 94, 92
7	68, 70	95, 94	99, 98
10	98, 99, 96	98, 102, 96	97, 96, 97
Mean	84	94	93
Standard deviation	±11	±14	±8

* The figure of 51 for dimethoate obtained by Laboratory 1 is a "rogue" result by Dixon's test and, if this figure is neglected, the mean ± standard deviation for dimethoate is 98 ± 4%.

difficulties associated with the quantitative gas - liquid chromatographic determination of these compounds. However, investigations in two laboratories indicated that the extraction and clean-up are basically sound for such compounds.

In the first two collaborative exercises reported for this method, the results for azinphos-methyl were lower than for other pesticides. This effect is ascribed to difficulties in the gas - liquid chromatography of azinphos-methyl associated with the high column temperature required. However, it is believed that, with the more successful gas-chromatographic systems used later with the modified Watts *et al.* method, this pesticide would be recovered adequately by using the Abbott *et al.* method.

It was concluded that the method could be regarded as established for the determination of malathion, dichlorvos, dimethoate, omethoate and parathion in carrots, beans, peas, apples, tomatoes and lettuces. The experience of the Panel suggests that the method would be widely applicable to the determination of many other non-polar and medium-polarity organophosphorus pesticides and to a wider range of fruits and vegetables. However, in these instances the method should be tested before use.

TABLE IV

RECOVERY OF OMETHOATE FROM APPLES, CARROTS, LETTUCES AND PLUMS BY THE MODIFIED ABBOTT *et al.* METHOD (APPENDIX I)

Crop	Laboratory	Recovery, % (0.4 mg kg^{-1})
Apples	2	96, 90
	4	87, 85
	6	95, 90
	7	81, 77
	10	72, 91
	Mean	86
	Standard deviation	±8
Carrots	2	110, 107
	4	84, 90
	6	90, 95
	7	94, 90
	10	90, 97
	Mean	95
	Standard deviation	±8
Lettuces	2	98, 91
	4	98, 100
	5	81, 85
	6	91, 89
	7	90, 91
	10	75, 81
	Mean	89
	Standard deviation	±8
Plums	2	83, 74
	4	80, 84
	5	87, 83
	6	85, 90
	7	83, 80
	10	94, 93
	Mean	85
	Standard deviation	±5
All crops	Mean	88
	Standard deviation	±8

Modified Watts *et al.* Method[5]

The modified Watts *et al.* method was also considered as a general procedure. The National Food Institute version of the method (Appendix II) was adapted for use by the Panel by substitution of a 1+1 mixture of toluene - acetone for the more toxic benzene in the elution mixture. The results in Table V show its applicability to dichlorvos, dimethoate and malathion in lettuces. A further study of the method was then undertaken for these three pesticides in carrots, peas, apples, tomatoes and plums and for parathion in carrots and tomatoes, with satisfactory results (Table VI). The reagent and crop blanks were satisfactory (<0.05

mg kg^{-1}). The method was also used satisfactorily for the recovery of omethoate from apples, carrots, lettuces and plums (Table VII). Following a review in one laboratory of columns for gas - liquid chromatography of azinphos-methyl, more satisfactory conditions were devised and a further collaborative study was carried out on the recovery of this pesticide from apples. The results are given in Table VIII.

TABLE V

RECOVERY OF DICHLORVOS, DIMETHOATE AND MALATHION FROM LETTUCES BY THE MODIFIED WATTS *et al.* METHOD (APPENDIX II)

	Recovery, %		
Laboratory	Dichlorvos (1 mg kg^{-1})	Dimethoate (2 mg kg^{-1})	Malathion (2 mg kg^{-1})
2	91, 88	86, 98	77, 79
4	—	78, 91	88, 92, 98, 99
5	96, 101	98, 100, 106	95, 94, 92
6	97, 97	95, 96	90, 92
7	91, 87	98, 101	98, 107
8	75	90	91
9	94, 96	75, 78, 78	95, 101
10	108, 96, 103	104, 98, 105	100, 98, 103
Mean	94	94	94
Standard deviation	±8	±9	±7

As with the Abbott *et al.* method, further investigations of this method for such compounds as demeton-*S*-methyl sulphoxide and sulphone have been hampered by difficulties over the adequate quantitative gas - liquid chromatography of these compounds. However, investigations indicated that the extraction and clean-up are basically sound for them.

The Panel concluded that the method could be regarded as established for the determination of malathion, dichlorvos, dimethoate, omethoate, azinphosmethyl and parathion in lettuces, carrots, peas, apples, tomatoes and plums. The experience of the Panel suggests that the method would be widely applicable to the determination of many other non-polar and medium-polarity organophosphorus pesticides and to a wider range of fruits and vegetables. Again, however, in these instances the method should be tested before use.

Sissons and Telling Methods[4]

The Panel also examined the methods of Sissons and Telling, which were modified to some extent during the course of the investigations. The methods, which are applicable to a wide range of both organochlorine and organophosphorus pesticide residues, are used in one instance for relatively non-polar species and in the other for those which are more polar. Further modification of the latter method is needed for such oxons as omethoate. The Panel obtained acceptable results for the method for non-polar species, but the results for dichlorvos using the method for polar species showed a much wider spread between laboratories. These results will be published elsewhere.

Gas - Liquid Chromatography

The Panel considered that some guidance should be given for preparing suitable gas - liquid chromatography columns, and this is included in Appendix III. In one collaborative study a recommended column of 5% OV-17 plus 0.02% Epikote 1001 on 80–100-mesh Gas-Chrom Q was prepared by individual laboratories from common materials by an agreed method and compared with the laboratories' own columns with respect to their behaviour in the chromatography of dichlorvos, malathion and dimethoate; the results obtained with the former column were only marginally, and not significantly, better.

In the studies described in this report results were obtained with both alkali flame ionisation and flame photometric detectors and the results given in the tables do not make any distinction between them. When the same solutions were examined by both detectors, a mean value is given. A statistical treatment of the results obtained by the two detectors for the same solutions showed that there is no significant difference ($P \leqslant 0.05$). A similar conclusion has been arrived at by other workers.[6]

TABLE VI

RECOVERY OF DICHLORVOS, DIMETHOATE, MALATHION AND PARATHION FROM CARROTS, PEAS, APPLES, TOMATOES AND PLUMS BY THE MODIFIED WATTS *et al.* METHOD (APPENDIX II)

Crop	Laboratory	Recovery, % Dichlorvos (0.5 mg kg^{-1})	Dimethoate (2 mg kg^{-1})	Malathion (2 mg kg^{-1})	Parathion (0.7 mg kg^{-1})
Carrots ..	2	95, 97	108, 111	100, 102	84, 93
	5	98, 98	92, 90	100, 102	96, 105
	6	79, 76	93, 98	94, 89	75, 69
	7	74, 66	99, 100	111, 105	92, 100
	10	103, 94	92, 94	92, 96	49, 101
	Mean	88	98	99	91
	Standard deviation	±13	±7	±7	±12
Peas	1	65, 93	89, 98	88, 108	—
	6	82, 79	104, 104	100, 94	
	8	84, 84	82, 90	98, 79	
	10	96, 92	99, 98	95, 98	
	11	84, 84	103, 86	80, 84	
	Mean	84	95	92	
	Standard deviation	±9	±8	±9	
Apples ..	4	78, 83	84, 94	89, 96	—
	8	71, 74	96, 90	96, 93	
	10	78, 82	92, 93	94, 98	
	11	94, 71	92, 96	83, 71	
	Mean	79	91	90	
	Standard deviation	±8	±5	±9	
Tomatoes ..	2	98, 113	107, 110	99, 103	81, 92
	5	102, 99	100, 102	96, 98	103, 104
	6	85, 82	70, 80	99, 95	87, 87
	7	89, 81	98, 91	106, 94	88, 89
	10	101, 85	94, 86	92, 84	100, 78
	Mean	94	94	97	91
	Standard deviation	±11	±12	±6	±9
Plums ..	1	92, 90	100, 102	99, 101	—
	2	94, 99	97, 107	93, 99	
	4	79, 80	96, 96	96, 102	
	5	99, 95	84, 102	96, 108	
	7	97, 85	111, 114	110, 102	
	10	88, 67	108, 96	107, 96	
	Mean	89	101	101	
	Standard deviation	±10	±8	±5	
All crops ..	Mean	87	96	96	91
	Standard deviation	±10	±9	±8	±11

APPENDIX I

Abbott *et al.* Method for Determination of Organophosphorus Pesticide Residues in Fruits and Vegetables

Principle

The finely chopped representative sample is mixed with anhydrous sodium sulphate and extracted with acetonitrile. The extract is diluted with a large volume of aqueous sodium sulphate solution and the pesticides, and some other materials, are extracted into chloroform. The chloroform solution is dried and concentrated for gas - liquid chromatography.

TABLE VII

RECOVERY OF OMETHOATE FROM APPLES, CARROTS, LETTUCES AND PLUMS BY THE MODIFIED WATTS *et al.* METHOD (APPENDIX II)

Crop	Laboratory	Recovery, % (0.4 mg kg^{-1})
Apples	1	89, 103
	2	107, 110
	6	84, 76
	7	80, 82
	10	93, 96
	Mean	92
	Standard deviation	±12
Carrots	1	76, 95
	2	107, 110
	6	100, 92
	7	97, 97
	10	89, 98
	Mean	96
	Standard deviation	±9
Lettuces	1	80, 73
	2	104, 102
	4	88, 112
	5	86, 86
	6	77, 76
	7	88, 95
	10	87, 91
	Mean	89
	Standard deviation	±11
Plums	1	77, 74
	2	103, 98
	4	75, 86
	5	102, 95
	6	72, 72
	7	98, 102
	10	92, 88
	Mean	88
	Standard deviation	±12
All crops	Mean	91
	Standard deviation	±11

TABLE VIII

RECOVERY OF AZINPHOS-METHYL FROM APPLES BY THE MODIFIED WATTS *et al.* METHOD (APPENDIX II)

Laboratory	Recovery, % (0.5 mg kg^{-1})
1	72, 71
2	96, 96
4	101, 114
5	95, 98
6	81, 90
7	99, 105
10	86, 93
Mean	93
Standard deviation	±12

Reagents

Solvents should be checked for interference in gas - liquid chromatography before use.
Acetone. Analytical-reagent grade.
Acetonitrile. Distol grade or equivalent.
Chloroform. Distol grade or equivalent.
Sodium sulphate, granular, anhydrous. Analytical-reagent grade.
Sodium sulphate, 2.5% aqueous solution. Analytical-reagent grade.
Propylene glycol, 50% solution in acetone. Re-distil the glycol, if necessary.
Pure pesticides. For standard solutions.
Nitrogen. Oxygen-free; carrier gas for gas - liquid chromatography.
Oxygen. For gas - liquid chromatography detector.
Hydrogen. For gas - liquid chromatography detector.

Apparatus

Blender.
Separating funnel. Capacity 1 l.
Cotton-wool.
Anti-bumping granules.
Filter funnel. About 15 cm diameter.
Calibrated flasks.
Micro-Snyder condenser.
Chromatography column. About 300 × 15 mm.
Kuderna-Danish evaporator. Fitted with a 10-ml graduated tube, but no fractionating column.
Steam-bath.
Water-bath.
Gas chromatograph with recorder and detector suitable for determining organophosphorus compounds.

Procedure

Weigh 20 g of a finely chopped representative sample into a blender cup and mix 30 g of anhydrous sodium sulphate, or sufficient to give a friable mixture, with the sample. Add 50 ml of acetonitrile and blend at high speed for 3 min. Decant the extract through a filter-funnel containing a small cotton-wool plug into a 1-l separating funnel containing 500 ml of 2.5% sodium sulphate solution. Repeat the extraction and decanting procedures twice more each time with 50 ml of acetonitrile and the same sodium sulphate solution. Mix the contents of the separating funnel and extract the aqueous solution with 50 ml of chloroform. Ensure the complete separation of the two layers. Run the chloroform layer down a 10-cm column of granular anhydrous sodium sulphate contained in a chromatography column into a Kuderna-Danish evaporator fitted with a 10-ml graduated tube. Repeat the chloroform extraction and drying procedure twice more, each time with 50 ml of chloroform. Add 0.1 ml of propylene glycol solution as a keeper, add a few anti-bumping granules and reduce the volume of the chloroform to about 2 ml on a steam-bath. The level of the liquid in the flask should always be above the level of water in the bath. Remove the apparatus and allow it to cool to room temperature before removing the still head. Rinse the evaporator with 3–5 ml of acetone and allow 5 min for drainage. Remove any moisture collected around the outside of the joint of the graduated tube and evaporator with a cloth. Separate the tube from the evaporator and rinse the male joint with 1 ml of acetone. Fit a micro-Snyder condenser and carefully remove the solvent by boiling over a steam-bath until only 0.5 ml remains in the bottom of the tube. Remove the apparatus from the steam-bath, wait about 1 min, then carefully run 0.5 ml of acetone into the top of the micro-Snyder tube and allow the acetone to drain down into the graduated tube. Repeat the latter acetone washing twice and remove the micro-Snyder tube. Place the graduated tube into a water-bath at 30 °C and blow a stream of dry air on to the surface of the extract until a small constant volume remains. Remove the graduated tube and make up to a suitable volume with acetone for gas - liquid chromatography.

Modified Procedure

When analyses are being carried out for more polar types of insecticide, such as omethoate, use 100 ml of 10% instead of 500 ml of 2.5% sodium sulphate solution for dilution of the acetonitrile extract and extract the diluted extract with 100 ml of chloroform in the first extraction. Then proceed as above.

APPENDIX II

Modified Watts *et al.* Method for Determination of Organophosphorus Pesticide Residues in Fruits and Vegetables

Principle

The finely divided sample is extracted with ethyl acetate and the extract cleaned up by chromatography on a column of activated charcoal - magnesium oxide - Celite. The organophosphorus insecticides are eluted from the column with ethyl acetate - acetone - toluene and examined by gas - liquid chromatography.

Reagents

Solvents should be checked for interference in gas - liquid chromatography before use.

Ethyl acetate. Analytical-reagent grade.

Toluene. Analytical-reagent grade.

Acetone. Analytical-reagent grade.

Sodium sulphate, anhydrous. Analytical-reagent grade.

Activated charcoal. Add 225 g of Nuchar C-190N to 1.2 l of an aqueous solution containing 50% of ethanol and 10% of concentrated hydrochloric acid. Reflux the mixture for 1 h, collect the charcoal on a Büchner funnel and wash with de-ionised or distilled water until a test-paper shows only a trace amount of acid to be present. Further wash the charcoal with acetone and aspirate it until it is nearly dry. Air-dry it until it is odourless (2–3 d) and finally dry it in a porcelain dish at 130 °C for 48 h. Store the purified charcoal in a tightly stoppered bottle.

Magnesium oxide. Mix 150 g of magnesium oxide (BDH Chemicals, for chromatographic adsorption analysis, or equivalent) with sufficient water just to cover it in a 1-l conical flask. Heat the mixture, with shaking, on a steam-bath for 30 min, collect the magnesium oxide on a Büchner funnel and dry it at 110 °C for 1 d. Sieve the dry powder through a 60- or 72-mesh screen (it absorbs about 10% of water in this procedure). Store it in a well stoppered bottle.

Celite 545.

Chromatographic mixture. Mix 20 g of activated charcoal, 40 g of magnesium oxide and 80 g of Celite 545 on a mechanical roller for about 1 h.

Propylene glycol. Re-distil if necessary. Prepare a 50% solution in acetone.

Pure pesticides.

Apparatus

Blender. With means of cooling the cup.

Chromatography column. 180 × 18 mm i.d., having a 150-ml capacity reservoir at its upper end. The lower end is constricted to about 4–5 mm i.d. but without a tap. A means of applying pressure or suction to the chromatography column should be available.

Cotton-wool.

Filter-funnel. About 100–150 mm diameter.

Water-bath.

Evaporator.

Calibrated flasks.

Gas chromatograph with recorder and detector suitable for determining organophosphorus compounds.

Procedure

Place 50 g of a finely chopped sample in a blender cup and add 250 ml of ethyl acetate. Add 40 g of anhydrous sodium sulphate immediately before the blender is started. With vegetables that have a high water content, increase the amount of sodium sulphate to 100 g.

Blend for 5 min at a low speed and then for 5 min at high speed, ensuring that the mixture becomes no more than slightly warm (heating can be minimised by using a water-cooled blender cup or by removing the cup and contents to ice-cold water between the two extraction steps). Filter the residue through cotton-wool. Insert a plug of cotton-wool in the bottom of the chromatography column and fill it, preferably under suction, with 10 g of the chromatographic mixture. Cover the top of the absorbent with a small plug of cotton-wool. Pour 50 ml of 1 + 1 + 2 ethyl acetate - acetone - toluene on to the column and force it through with slight pressure or under suction. When the solvent level reaches the top of the column, discard this effluent. Add 125 ml of the sample extract to the column and adjust the flow-rate to about 5–6 ml min^{-1} of eluate using air or nitrogen pressure or suction. (If the column is run too slowly, recovery is incomplete.) Then elute the column with 150 ml of 1 + 1 + 2 ethyl acetate - acetone - toluene until the column runs dry. Collect the total volume of liquid passing through the column from the extract and elution. Add 0.1 ml of propylene glycol solution to the eluate as a keeper and concentrate to about 1 ml under vacuum. Make up to a suitable volume with acetone for gas - liquid chromatography.

APPENDIX III

Gas - Liquid Chromatography

Glass columns, using on-column injection or glass-lined injection ports, should be used for the gas chromatography of cleaned-up solutions of organophosphorus pesticide residues. The columns should be 1–2 m long (preferably 1 m unless better resolution is required) and 2–4 mm i.d. (preferably 2 mm.) The pipework from the end of the column to the detector should be as short as possible and should be glass-lined. Chromatography is usually improved by silanising all glass parts, including glass-wool plugs.

Packings that have given satisfactory results in more than one laboratory for the work reported here are 3 or 5% OV-17 with Epikote 1001 (0.02%) on 60–80- or 80–100-mesh Gas-Chrom Q and 1.3% Apiezon L on 80–100-mesh acid-washed Chromosorb G. For omethoate, 5% Carbowax 20M on 80–100-mesh Gas-Chrom Q or 5% DEGS on 80–100-mesh Gas-Chrom Q have been useful. The temperature of the column will depend on the pesticide: 110–130 °C was found to be suitable for dichlorvos and 180–210 °C was suitable for most others. For azinphos-methyl, 3% OV-17 on 80–100-mesh Gas-Chrom Q or Chromosorb W at 250 °C has been used successfully in three laboratories; 2% Apiezon L on a suitable support at about 240 °C has also been found to be satisfactory.

It is often necessary to treat columns by injection of such formulations as Silyl 8 and usually to pre-treat the columns with injections of crop extracts from blanks and also solutions of standards before injecting solutions of analytical samples in order to obtain acceptable reproducibility. The injection of crop extracts should be continued from time to time between the injections of calibration solutions as extracts affect the response to pesticides.

In general, alkali flame ionisation or flame photometric detectors are to be preferred, but for a few compounds electron-capture detectors may be sufficiently selective.

A suitable method for preparing a column of 5% OV-17 plus 0.02% Epikote 1001 on 80–100-mesh Gas-Chrom Q has been found to be as follows.

1. Weigh 0.5 g of OV-17 and 0.002 g of Epikote 1001 and dissolve both in about 25 ml of chloroform (it is difficult to see the OV-17 once it has been wetted with solvent) in a 100-ml glass beaker.
2. Pour in 10 g of Gas-Chrom Q (80–100 mesh), mix and leave to stand for 10 min with occasional swirling.
3. Holding the beaker in a warm water bath (about 40 °C) in a fume cupboard, blow off the solvent gently with a stream of air or nitrogen while constantly stirring slowly with a micro-spatula. Take great care not to crush the particles against the sides of the beaker.
4. When a dry powder remains and there is no odour of solvent, place it on a watch-glass, cover with a sheet of clean paper and air dry it overnight.
5. Pack into a 1 or 2 m × 2 mm i.d. silanised glass column using a suitable vacuum source and silanised glass-wool plugs. Secure the column in the instrument without connecting

the detector and, with a carrier gas flow-rate of about 40 ml min^{-1}, condition it overnight at 265 °C.

6. Reduce the column-oven temperature to about 250 °C for azinphos-methyl, 210 °C for malathion, parathion and dimethoate or 130 °C for dichlorvos. Connect the detector, light the flame and inject 5–10 μl of crop extract before starting the analysis.

It should be made clear that the above are not necessarily the best gas - liquid chromatographic conditions for the analysis of organophosphorus pesticide residues, but they have been used satisfactorily in two or more of the laboratories that took part in this work.

APPENDIX IV

Membership of the Panel

The following laboratories, represented by the workers named, contributed to the work of the Panel: Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food (M. P. Quick); Cyanamid of Great Britain Ltd. (P. Russell); County of Avon Scientific Services (G. J. Dickes); East Malling Research Station (J. G. Allen, R. P. Tew); Laboratory of the Government Chemist, Department of Industry (D. C. Hunt, G. H. J. Merson, B. H. Tait); Kent County Analytical Laboratory (R. E. S. Andrews, R. Fawcett); National Food Institute, Demark (F. Bro-Rasmussen, K. Gram-Jensen, K. Voldum-Clausen); Pest Infestation Control Laboratory, Ministry of Agriculture, Fisheries and Food (P. M. Brown, J. S. Clarke, D. F. Horler, D. G. Rowlands); Plant Pathology Laboratory, Ministry of Agriculture, Fisheries and Food [A. R. C. Hill, N. A. Smart (Secretary)]; Somerset County Council, County Analyst's Laboratory [W. Cassidy (Chairman)]; Tropical Products Institute, Ministry of Overseas Development (J. Cox, L. Donegan); and Unilever Research (P. Brawn, G. M. Telling).

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Analytical Methods Committee

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

Determination of Sulfanitran in Animal Feeds and Pre-mixes

Keywords: *Sulfanitran determination*; *animal feeds*; *spectrophotometry*

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

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. J. Markland (Chairman), Mr. R. J. Anderson, Mr. F. Bailey, Mr. A. G. Croft, Mr. C. E. Dodd, Mr. R. Fawcett, Dr. K. Field, Mr. R. S. Hatfull, Mr. I. D. Lee, Mr. D. H. Mitchell and Mr. J. A. Stubbles, with Mr. P. W. Shallis as Secretary.

Introduction

Sulfanitran [4′-(4-nitrophenylsulphamoyl)acetanilide] is a coccidiostat used in poultry feed at a recommended level of inclusion of about 300 mg kg^{-1}. The use of this drug in the United Kingdom for the purpose of animal health is restricted to veterinary prescription.

Experimental

The Sub-Committee began its work on sulfanitran at the time when the drug was under consideration by the EEC for acceptance into Annex I of the list of permitted drugs. The published official method of the AOAC[1] was first investigated and some members found the method to be reasonably satisfactory, whereas others found that the calibration graphs were neither linear nor reproducible. It was also reported that the final coloured complex, formed by coupling the deacetylated and diazotised drug with *N*-2-aminoethyl-1-naphthylamine dihydrochloride, was not stable in aqueous solution. One laboratory suggested that more stable and reproducible calibration graphs could be obtained by extraction of the coloured complex into an organic solvent, in which the complex was stable. This method was examined collaboratively on various feeds medicated at levels of 50 and 300 mg kg^{-1}; the results are shown in Table I. A few of the unmedicated feeds examined during the course of the Sub-Committee's work exhibited apparent sulfanitran contents of up to 30 mg kg^{-1}; no reason for this was found. A programme of work designed to examine the cause of the high apparent sulfanitran contents of some feeds was arranged but was not carried out as at that time it was learned that the EEC was not going to accept sulfanitran as a permitted drug. As the drug was, however, still permitted for use on veterinary prescription within the UK, the Sub-Committee felt that it would be useful to have available a method for its determination in animal feeds. It was considered that the method the Sub-Committee had been examining was adequate for the purpose; full details are given in the Appendix.

Recommendation

The Sub-Committee recommends that the method given in the Appendix should be used for the determination of sulfanitran in complete animal feeds and pre-mixes.

TABLE I

DETERMINATION OF SULFANITRAN IN DIFFERENT FEEDS BY THE RECOMMENDED METHOD

Laboratory	Sulfanitran added/ mg kg^{-1}	Apparent sulfanitran found/ mg kg^{-1}	Feed blank*/ mg kg^{-1}	Sulfanitran recovered/ mg kg^{-1}	Recovery, %
A	59	76.2	18.3	57.9	98.1
		76.2		57.9	98.1
		76.0		57.7	97.8
B	50	51.0	0	51.0	102.0
		51.0		51.0	102.0
		48.5		48.5	97.0
C	50	56.0	14.0	42.0	84.0
		46.0		32.0	64.0
D	50	46.6	0	46.6	93.2
		46.6		46.6	93.2
E	52.7	58.0	14.0	44.0	83.5
		44.0	28.0	16.0	30.4
		48.0	10.0	38.0	72.1
		54.0	11.8	42.2	80.1
		52.0	11.3	40.7	77.2
A	297	318	18.3	299.7	100.9
		318		299.7	100.9
		320		301.7	101.6
B	300	308.0	0	308.0	102.7
		300.0		300.0	100.0
		320.0		320.0	106.7
C	300	296	14.0	282.0	94.0
		314		300.0	100.0
D	500	492	0	492	98.4
		484		484	96.8
E	316	304	14.0	290.0	91.8
		322	28.0	294.0	93.0
		310	10.0	300.0	94.9
		328	11.8	316.2	100.1
		315	11.3	303.7	96.1

* Several different blank meals were used in the work, only some of which exhibited high apparent sulfanitran contents that gave rise to very low recoveries.

APPENDIX

Recommended Method for the Determination of Sulfanitran in Animal Feeds and Pre-Mixes

Scope and Field of Application

The method is for the determination of the amount of sulfanitran in complete feedingstuffs and pre-mixes. Any drug capable of being diazotised and coupled with *N*-2-aminoethyl-1-naphthylamine dihydrochloride under the conditions of the method will interfere.

Principle

Sulfanitran is extracted from the feed with hot methanol. The extract is cleaned-up by co-precipitation of certain interfering substances with zinc hydroxide followed by filtration. The filtrate is acidified with hydrochloric acid and then boiled for 1 h in order to deacetylate the drug and to remove the methanol. After diazotisation and coupling with *N*-2-aminoethyl-1-naphthylamine dihydrochloride, the coloured complex is extracted into a mixture of butan-1-ol and hexane and its absorbance measured at the maximum at about 545 nm.

Reagents

Methanol.

Zinc sulphate solution, 10 g l^{-1}.

Hydrochloric acid (*sp. gr.* 1.18).

Sodium nitrite solution, 1 g l^{-1}. This solution should be stored in a refrigerator and should not be used when more than 1 week old.

Ammonium sulphamate solution, 5 g l^{-1}.

Coupling reagent. An aqueous solution containing 1 g of *N*-2-aminoethyl-1-naphthylamine dihydrochloride per litre. Store this solution in a dark brown bottle.

Sodium hydroxide solution, 1 N.

Solvent mixture. Mix 4 parts by volume of butan-1-ol with 1 part of spectroscopically pure hexane.

Sodium chloride.

Standard substance. Pure sulfanitran.

Sulfanitran standard solutions. Weigh out, to the nearest 1 mg, 0.100 g of the standard substance and transfer it to a 1 000-ml calibrated flask. Dissolve the solid in a small volume of 1 N sodium hydroxide solution and dilute to the mark with water (stock solution, 100 μg ml^{-1} of sulfanitran). By pipette transfer 10 ml of the stock solution to a 100-ml calibrated flask and dilute to the mark with water (working standard solution, 10 μg ml^{-1} of sulfanitran).

Apparatus

Spectrophotometer, with 10-mm *cells.*

Procedure

Extraction of sulfanitran

Weigh, to the nearest 0.01 g, approximately 5 g of the feed sample, transfer it to a 100-ml calibrated flask and add 75 ml of methanol. Place the flask in a water-bath at 60 $\pm$ 1 °C for 20–30 min, shaking the flask occasionally. Remove the flask from the water-bath, allow the flask and contents to cool to room temperature, and then dilute the contents to the mark with methanol. Shake the flask vigorously and then set it aside for 40 min in order to allow the feed particles to settle. By means of a pipette transfer 25.0 ml of the supernatant methanol extract to a 50-ml calibrated flask. To the contents of the flask add 10 ml of water, 5 ml of zinc sulphate solution and 3–5 drops of 1 N sodium hydroxide solution; keep this solution near to neutrality. Place the flask in a bath of boiling water for 2 min (or leave it at room temperature for 5 min) to assist the precipitation of interfering substances. If necessary cool the flask and contents, dilute to the mark with water, mix thoroughly and filter through a Whatman No. 42, or equivalent, filter-paper. Discard the first 5 ml of filtrate.

Deacetylation of sulfanitran

By means of a pipette transfer 10.0 ml of the filtrate to a 50-ml calibrated flask containing 8 ml of water and 0.5 ml of concentrated hydrochloric acid. Place the flask in a bath of boiling water for 1 h, with frequent shaking of the flask during the first 15 min. Cool the flask and contents to room temperature and dilute to the mark with water. If the solution is turbid, clarify it by spinning it in a centrifuge.

Colour development

By use of a pipette transfer separate 5.0-ml portions of the deacetylated solution of the drug into each of two centrifuge tubes. To one tube, to be used as the blank, add 1 ml of water and 0.5 ml of the coupling reagent. To the contents of the other tube add 0.5 ml of sodium nitrite solution and mix well. After 3 min add 0.5 ml of ammonium sulphamate solution and again mix well. After a further 2 min add 0.5 ml of the coupling reagent, mix well and set the tube aside for 10 min.

To the contents of both tubes add about 2 g of sodium chloride and 5.0 ml of the solvent mixture. Next shake the tubes until all of the sodium chloride has dissolved and the colour is contained in the organic layer. Spin the tubes in a centrifuge and then, with a Pasteur

pipette, transfer the coloured organic layer from both tubes to separate 10-mm cells. Measure the absorbances of both solutions at the maximum at a wavelength of about 540 nm against the solvent mixture. Subtract the reading for the blank from the reading for the diazotised sample extract to give the corrected absorbance for the sample.

Calibration graph

Transfer to separate 50-ml calibrated flasks 0-, 1-, 2-, 4-, 6-, 8- and 10-ml portions of the working standard solution and to the contents of each flask add 0.5 ml of concentrated hydrochloric acid; adjust the volume in each flask with water to approximately 15 ml. Place the flasks in a bath of boiling water for 1 h, then remove the flasks from the bath, cool them to room temperature and dilute the contents of each to the mark with water. By means of a pipette transfer a 5.0-ml portion from each tube to a separate centrifuge tube and proceed with each as described above under *Colour development*. The centrifuge tubes contain, respectively, 0, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 μg of sulfanitran.

Plot a graph of absorbance against micrograms of sulfanitran.

Calculation of results

Calculate the sulfanitran content of the sample from the expression

$$\text{Sulfanitran/mg kg}^{-1} = \frac{200 \times C}{M}$$

where C represents the micrograms of sulfanitran equivalent to the corrected absorbance for the sample read from the calibration graph and M is the mass of sample taken, in grams.

Determination of the Sulfanitran Content of Pre-mixes

Carry out the determination of sulfanitran in pre-mixes in the same way as described for feedingstuffs, but with the following modifications. Adjust the mass of sample taken and the various dilutions effected during the extraction and deactylation so that the final coloured solution contains 3–8 μg of sulfanitran. Refer the corrected absorbance to the calibration graph, but in the calculation of the results take into account any additional dilutions employed.

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SHORT PAPERS

Improved Method for the Determination of Pyrimethamine in Poultry and Rabbit Feeding Stuffs by Gas - Liquid Chromatography

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Keywords: Pyrimethamine determination; animal feeding stuffs; gas - liquid chromatography

Pyrimethamine [5-*p*-chlorophenyl-6-ethylpyrimidine-2,4-diamine] is used in rabbit and poultry feeds at levels of 5–7.5 mg kg^{-1} for the prevention of coccidiosis. It is normally used in the presence of sulphaquinoxaline, with which it exerts a synergistic effect.

A spectrophotometric method for the determination of pyrimethamine in animal feeds was described by Garber *et al.*[1] This method is based on the formation of a complex betweeen pyrimethamine and bromocresol green in aqueous solution. In our experience, this method is subject to interference from certain ingredients in some feeds, particularly grassmeal. The method for the determination of pyrimethamine in feeds by HPLC recently described by Cox and Sugden[2] allows determinations only to the nearest 1 mg kg^{-1}.

Methods for the determination of pyrimethamine in animal tissue have been described by Cala *et al.*[3] (gas - liquid chromatography), and Golinskii and Repin[4] (adsorption chromatography). However, neither of these methods is directly applicable to animal feeds.

Royere *et al.*[5] described a gas - liquid chromatographic method for the determination of pyrimethamine in animal feeds. The method involves the extraction of pyrimethamine with 50% hydrochloric acid followed by determination using an electron-capture detector. However, when the method was applied in this Laboratory to compound feeds it was not possible to resolve the pyrimethamine peak from those of co-extractives and difficulty was often experienced during the clean-up stages owing to the formation of emulsions. It was considered that the use of an organic solvent to reduce the amount of co-extracted material, coupled with a more thorough clean-up procedure, would permit satisfactory determinations by gas - liquid chromatography. In the Report of the Government Chemist for 1974[6] a modification to the method of Garber *et al.*[1] was described in which the extraction solvent was changed from benzene to methanol; the latter solvent has been retained for the method described in this paper.

Experimental

Apparatus

A gas - liquid chromatograph fitted with a nickel-63 electron-capture detector (Pye, Model GCV, fitted for on-column injections) was used, with a glass column (1 m × 4 mm) containing 10% *m/m* Versamid 900 on Diatomite CLQ (80–100 mesh). The carrier gas was nitrogen (oxygen free) at a flow-rate of 50 ml min^{-1} and the purge gas was also nitrogen (oxygen free) at a flow-rate of 30 ml min^{-1}. The column was maintained at 230 °C, the injector port at 250 °C and the detector at 300 °C.

A sintered-glass micro-filter, porosity 1, i.d. 10 mm (available from Scientific Supplies Co. Ltd., London), and a rotary vacuum evaporator were used.

Reagents

Sodium sulphate, anhydrous. Heated overnight at 350 °C.

Chloroform.
Hexane.
Methanol.
Hydrochloric acid, 0.1 M.
Sodium hydroxide solution, 1 M.
Internal standard solutions.

Stock solution. Dissolve 40 mg of 4-chloropyrimidine-2,6-diamine in methanol and dilute to 100 ml with methanol.

Solution A. Dilute 4 ml of the stock solution to 100 ml with methanol (1 ml of solution contains 16 μg of 4-chloropyrimidine-2,6-diamine).

Solution B. Dilute 10 ml of the stock solution to 100 ml with methanol (1 ml of solution contains 40 μg of 4-chloropyrimidine-2,6-diamine).

Pyrimethamine standard solution. Weigh, to the nearest 0.1 mg, 40 mg of pure pyrimethamine, dissolve it in methanol and make up to 100 ml with methanol. Dilute 10 ml of this solution to 100.0 ml with methanol (1 ml of solution contains 40 μg of pyrimethamine).

Procedure

Weigh, to the nearest 0.01 g, approximately 30 g of the finely divided and mixed sample (previously ground to pass a 1-mm sieve) and transfer it into a 500-ml conical flask. Add 200 ml of methanol, stopper the flask firmly and shake it mechanically for 60 min. Allow the contents of the flask to settle and filter them through a Whatman No. 541 filter-paper.

Transfer 100 ml of the filtrate into a 250-ml conical flask and evaporate it to dryness on a water-bath at 70 °C under a stream of nitrogen (the use of a rotary vacuum evaporator is not recommended). Add 10 ml of 0.1 M hydrochloric acid and 10 ml of hexane to the flask, swirl to dissolve the residue and transfer the contents into a 100-ml separating funnel. Rinse the flask with a mixture of 10 ml of 0.1 M hydrochloric acid and 10 ml of hexane and finally with 5 ml of 0.1 M hydrochloric acid. Combine all the washings in the separating funnel and shake for 1 min. Allow the phases to separate and run off the acidic layer into a second 100-ml separating funnel. Add 15 ml of hexane to the acidic layer, shake, allow the phases to separate and run off the acidic layer into a third 100-ml separating funnel. To the hexane remaining in each of the first two separating funnels add 5 ml of 0.1 M hydrochloric acid, shake, allow the phases to separate and run off the acidic layer into the third separating funnel. Finally, wash the acidic solution in the third separating funnel with two 10-ml portions of chloroform and discard the chloroform.

Add 5 ml of 1 M sodium hydroxide solution to the acidic solution and extract the mixture with three 15-ml portions of chloroform. Run the extracts through a sintered-glass microfilter, previously filled to a height of 50 mm with sodium sulphate, into a 100-ml round-bottomed flask. Wash the column with two 5-ml portions of chloroform and add the washings to the flask.

Remove the chloroform under reduced pressure on a rotary vacuum evaporator at 30 °C and dissolve the residue in 10.0 ml of internal standard solution A. Inject 5 μl of this solution on to the chromatographic column. Measure the heights of the pyrimethamine and internal standard peaks.

Calibration

Transfer 2-, 4-, 6-, 8- and 10-ml aliquots of the diluted pyrimethamine standard solution into a series of 25-ml calibrated flasks, add 10.0 ml of internal standard solution B to each flask, dilute to the mark with methanol and mix (these solutions contain 3.2, 6.4, 9.6, 12.8 and 16.0 μg ml^{-1} of pyrimethamine, respectively). Inject 5 μl of each solution on to the chromatographic column and measure the heights of the pyrimethamine and internal standard peaks.

Calculations

Calculate the peak-height ratio of pyrimethamine to internal standard for all solutions. Plot a graph of peak-height ratio against concentration of pyrimethamine (micrograms per millilitre) in the standard solutions and from the graph determine the concentration of pyrimethamine in the feed solution as injected.

The amount of pyrimethamine in the feed in milligrams per kilogram is given by the formula $20C/M$, where C is the concentration of pyrimethamine (micrograms per millilitre) in the feed solution as injected and M is the mass of sample in grams.

Retention Times

The retention time of the internal standard was 5 min and that of pyrimethamine was 15 min.

Results and Discussion

Pyrimethamine was added at various levels to four poultry feeds, which were then analysed using the proposed method; the results are given in Table I. Recoveries were of the order of 96% and no interference from grassmeal or fishmeal was observed.

TABLE I

RECOVERY OF PYRIMETHAMINE FROM POULTRY FEEDS

Feed	Pyrimethamine/mg kg^{-1} Added	Found	Recovery, %
A*	5.1	4.9	96
	5.1	4.9	96
B	5.8	5.6	97
	5.8	5.4	93
C	5.3	5.2	98
	5.3	5.0	94
D	1.7	1.7	100
	3.7	3.7	100
	6.7	6.5	97
	7.1	6.4	90
D + 10% of fishmeal	7.2	6.9	96

* Feed A contained 10% of grassmeal.

Two feeds in pelleted form, typical of those available commercially, were obtained for examination. In addition to pyrimethamine, they contained sulphaquinoxaline (feed E) and amprolium, ethopabate and sulphaquinoxaline (feed F). These feeds were examined by the proposed method, the gas - liquid chromatographic method of Royere *et al.*,[5] the spectrophotometric method[1] and the high-performance liquid chromatographic method.[2] The results are given in Table II.

TABLE II

COMPARISON OF DETERMINATION OF PYRIMETHAMINE BY DIFFERENT METHODS

Feed	Pyrimethamine content/mg kg^{-1} Gas - liquid chromatography: This method	Gas - liquid chromatography: Royere *et al.*[5]	Spectrophotometric method[1]	HPLC method[2]
E	5.2	Not	4	5
	5.4	measurable	3	5
F	1.9	Not	2.7	2
	2.1	measurable		3
	2.0			2

The results shown in Table II confirm the observations made during the initial stages of the work that the drugs with which pyrimethamine is normally incorporated do not interfere in the proposed method.

Repeatability

The repeatability of the method was ascertained by performing replicate analyses of a feed medicated with 4.83 mg kg^{-1} of pyrimethamine. The mean of seven determinations was 4.60 mg kg^{-1} (95.3% recovery) with a standard deviation of 0.07 mg kg^{-1}.

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Determination of Iodine Using a Kinetic Method with an Iodide Ion-selective Electrode

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Keywords: Iodine determination; iodide ion-selective electrode; water analysis; kinetic method

A method for the determination of iodine is described, based on the reaction of acetone, iodine and potassium hydroxide as monitored with an iodide-selective electrode. This method is more sensitive than those previously described.[1–3]

Kinetic methods of analysis and ion-selective electrodes have recently been recognised as useful means for the determination of individual elements, catalysts, enzymes, substrates, inhibitors and activators.[4–9] In a previous paper Altinata and Pekin described the determination of molybdenum and tungsten by using an iodide-activity electrode.[10]

Nikitin and Kochkin[4] have determined iodine by using a kinetic method, based on the determination of the rate of development of turbidity in solution with time. Three readings were taken of the turbidity time (t_x) and they carried out similar measurements on two control solutions (t_1 and t_2). They calculated the concentration of the iodine used as a substrate for the reaction between acetone, iodine and potassium hydroxide by applying the equation given in the literature.

There are three steps in the reaction between acetone, iodine and potassium hydroxide. These partial reactions can be summarised as the following net reaction:

$$CH_3COCH_3 + 3I_2 + 4KOH \rightarrow CHI_3 + CH_3COOK + 3KI + 3H_2O$$

In the present work the tangent method of kinetic analysis was used and iodine was determined by the increase of iodide activity with time, using an iodide ion-selective electrode and an Orion Model 401 electrometer. When iodide activities are plotted against time, the slope of the linear part of the graph is proportional to the concentration of iodine.

Experimental

Apparatus

An electrometer (Model 401, Orion Research Inc., Cambridge, Mass., USA), a thermostat bath (Haake TY 41, Gebrüder Haake, Berlin, Germany), an iodide ion-selective electrode

(Orion, Model 94–53) and a single-junction reference electrode (Orion, Model 90–01) were used. The iodide electrode gave a response in the concentration range 1 to 5×10^{-8} M at pH 0–14. Maximum allowable concentrations of the interfering ions for the electrode, expressed as the ratio of the interfering ion concentration to the iodide concentration in the sample,[11] are as follows: chloride, 10^6; bromide, 5×10^3; cyanide, 0.4; and thiosulphate, 10^5. Sulphide must be absent.

The concentrations of interfering ions in the reaction mixtures were always below the maximum allowable concentrations.

Reagents

All reagents were of pro analisi grade, supplied by E. Merck, Darmstadt, Germany, and re-distilled and de-ionised water was used throughout the work.

Iodine solution. Prepared by dissolving 0.4 g of iodine and 0.8 g of potassium iodide in 1 l of water, then diluting 1 ml of this solution to 1 l with water.

Potassium iodide solution, 4.8×10^{-6} M. Prepared by dissolving 0.8 g of potassium iodide in 1 l of water, then diluting 1 ml of this solution to 1 l with water.

Potassium hydroxide solution, 1.8×10^{-2} M. The required amount was dissolved in water.

Acetone solution, 13.6×10^{-2} M. Acetone (99.5%) was diluted to 13.6×10^{-2} M with water.

Procedure

Before each experiment, the amounts of potassium hydroxide, potassium iodide and acetone solutions that were required to obtain the concentrations shown in Table I were mixed and diluted to 60 ml with re-distilled and de-ionised water. These solutions were allowed to reach thermal equilibrium in a constant-temperature vessel (20 ± 0.01 °C) and were used to adjust the initial iodide activity. This was found from a knowledge of the total ionic strength (I) and a graph of activity coefficient (γ) *versus* total ionic strength[11] (I). The iodide and reference electrodes were immersed in the solution in the vessel; the selector switch was adjusted and the pointer on the scale set to the initial iodide activity.

In each experiment the required amounts of acetone and potassium hydroxide solutions were mixed and diluted to 55 ml with water. These solutions were allowed to reach thermal equilibrium in the vessel, and then 5 ml each of the iodine and iodide solutions were added. The stop-watch was started as soon as the iodine and iodide solutions were added and the increase in iodide activity with time was recorded.

The slope of the initial linear part of the graph of activity *versus* time was determined (tan α).

The above procedure was repeated for solutions containing different amounts of iodine, keeping the concentration of iodide constant. A calibration graph of tan α *versus* iodine concentration was drawn.

Results

Table I shows the relationship between iodine concentration and tan α. Using these results a calibration graph was drawn and the range up to 7 μg l^{-1} was accepted as the analytical region.

TABLE I

DETERMINATION OF IODINE

Reaction conditions: temperature, 20 ± 0.01 °C; concentration of reagents, potassium iodide 0.4×10^{-6} M, potassium hydroxide 6×10^{-3} M, acetone 4.53×10^{-2} M; pH = 11.

Concentration of iodine/μg l^{-1}	Tan α
0.66	1.80
2.00	2.40
4.00	3.35
5.30	4.00
6.60	4.60
8.00	5.20
10.00	5.30
13.30	5.50
20.00	5.90
26.00	6.20
30.00	6.50

Discussion

In this method amounts of iodine as low as 0.6 $\mu g\ l^{-1}$ can be determined. The relative error of the method is $\pm$ 1%. This method is more sensitive than the original methods[1–3] and it also has advantages of simplicity and economy compared with other methods of equivalent sensitivity.

The iodine concentration in drinking water is very important. It is known that a concentration of iodine in drinking water below 3 $\mu g\ l^{-1}$ can cause goitre. Goitre is prevalent near İzmir and the iodine content of the drinking water in this area was determined and found to be below 0.6 $\mu g\ l^{-1}$. In control studies of the drinking water consumed in İzmir, the iodine concentration was found to be 4 $\mu g\ l^{-1}$. The proposed method has been accepted for use in a medical screening programme for the control of goitre.

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Determination of Diolefins in Olefinic Cracked Hydrocarbon Products: Determination of Model Compounds

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Keywords: *Diolefin determination*; *cracked hydrocarbons*; *methoxymercuration*; *spectrophotometry*; *thin-layer chromatography*

Olefins are important feed stocks for the production of petrochemicals, and are generally produced by cracking processes. The liquid hydrocarbons obtained in cracked products contain α- and internal monoolefins and diolefins, the diolefins being both conjugated and non-conjugated. Conjugated dienes can be determined by use of the maleic anhydride method.[1] No satisfactory chemical method exists for the determination of non-conjugated dienes so it is therefore desirable that one should be developed.

Methoxymercuration is often used for the determination of unsaturation[2] based on the following reaction:

$$R\text{–}CH{=}CH_2 + Hg(COOCH_3)_2 + CH_3OH \rightarrow \begin{matrix} R\text{–}CH\text{–}CH_2 \\ | \quad\quad | \\ H_3CO \quad HgOOCCH_3 \end{matrix} + CH_3COOH$$

The determination of such adducts of monoolefins by use of spectrophotometry has been reported by the present authors.[3] A method for determining the non-conjugated dienes by such adduct formation, which is based on the separation of the adducts by thin-layer chromato-

graphy, is described here. There is a large difference between the R_F values of the methoxymercurated products of mono- and diolefins, obviously as a result of the high degree of polarity imparted by the acetoxymercury function to the hydrocarbon chain. Thus, a monomethoxymercurated adduct derived from monoolefins is more polar than the parent olefin. Introduction of a second acetoxymercury group (*i.e.*, from a diolefin) increases the polarity much more so that the R_F value becomes 0.03–0.06, whereas that of the monolefin adduct is 0.79–0.87. This permits the diolefin adduct to be conveniently separated over the thin-layer chromatographic plate using a suitable developing solvent. The separated organomercurial adduct can be determined quantitatively by spectrophotometry after complexation with diphenylcarbazone.

When conjugated dienes are present the sample can be treated first with maleic anhydride in toluene prior to methoxymercuration. Only conjugated dienes react with the maleic anhydride to give the Diels - Alder adducts; these adducts do not interfere in the separation and determination of the non-conjugated dienes.

Experimental

Apparatus and Reagents

A Spectronic 20 (Bausch & Lomb) spectrophotometer was used for the colorimetric determination. Thin-layer chromatographic plates were coated with silica gel G (E. Merck); they had an area of 20 × 20 cm and a thickness of 0.25 mm. These plates were activated at 110 °C for 30 min before use.

All of the chemicals used were of analytical-reagent grade. Diphenylcarbazone in acetone (150 mg per 100 ml) was used to form the coloured complexes.

A mixture of acetone, ammonia solution (sp. gr. 0.89) and water (100 + 40 + 40 *V*/*V*) was used as the developing solvent.

Mixtures of monoolefins and non-conjugated dienes (Table II) were prepared in methanol while mixtures of conjugated and non-conjugated dienes (Table III) were prepared without any solvent.

Procedure

Approximately 0.5 g of sample, accurately weighed, was treated with mercury(II) acetate [olefin to mercury(II) acetate molar ratio, 1 : 1.2] and methanol (5 ml) in the presence of 1 drop of glacial acetic acid and 1 drop of water. The reaction was carried out for 2 h, with vigorous stirring, in the dark at 30 °C. After the completion of the reaction the reaction mixture was diluted with methanol to a volume of 50 ml. Three spots of this solution were applied with a microsyringe at distances of 8.5, 14.5 and 18 cm from one side of the thin-layer chromatographic plate (1, 2 and 3, respectively, in Fig. 1) and the chromatogram was developed in a closed jar. Only the last spot (3, Fig. 1) was sprayed with diphenylcarbazone solution while the remaining areas of the plate were covered to locate the diolefin and monoolefin adducts.

The silica gel specimens from three equal zones (dotted lines, Fig. 1) were scraped off* and collected in three stoppered measuring cylinders. The adsorbent from the first zone, where no sample was spotted, was used as a blank. Each of these adsorbent samples was treated with 0.5 ml of distilled water for 30 min, then with 0.5 ml of diphenylcarbazone solution, and each was kept in the dark for 10 min; 10 ml of acetone were then added and the mixture was shaken thoroughly and kept for 20 min for complete desorption of the adduct. The coloured suspensions were filtered through a sintered-glass funnel under mild suction and the silica gel was washed with fresh acetone so that the filtrate and washings made up a volume of 25 ml. The absorbances of these coloured solutions were determined against the blank at 560 nm and the concentration of mercury was calculated from a straight-line calibration graph that had been plotted earlier.

Samples containing conjugated dienes were first treated with powdered maleic anhydride (diene to maleic anhydride molar ratio, 1:1.2) in toluene (2 ml) for 1 h at 30 °C with thorough stirring and then methoxymercurated. The non-conjugated dienes were determined as described above.

* All of the spent solvents, reagents and the scrapings from the thin-layer chromatographic plate (after desorption) should be stored in a closed container and suitably disposed of.

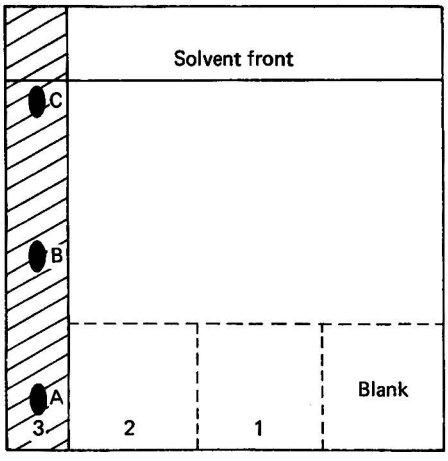

Fig. 1. Arrangement of thin-layer chromatographic plate. 1, 2 and 3, sample applied; ▨, area sprayed with diphenylcarbazone; A, diene adduct; B, excess of mercury(II) acetate; and C, monoolefin adduct; ⬚, zone scraped off.

Results and Discussion

A sharp separation of the methoxymercurated products of mono- and diolefins was observed on the thin-layer chromatographic plate with the developing solvent used. The values of R_F and $\lambda_{max.}$ for the coloured complexes are given in Table I. The monoolefin adduct moved almost with the solvent front (R_F 0.78–0.87) whereas the diene adduct moved very little (R_F 0.038–0.068). Mercury(II) acetate was found to be located between the two (R_F 0.35) and hence its presence in excess did not interfere in the determination of the dienes. The time required for the development was observed to be 45 min for a 10-cm distance. Other solvent mixtures, such as butan-2-one - ammonia solution - ethanol - propan-2-ol (10 + 7 + 4 + 1) tried earlier by Braun and Vorendohre[4] or propan-1-ol - triethylamine - water (50 + 25 + 25) used by Prey *et al.*,[5] required more time for development and still did not result in effective separation of the methoxymercurated adducts of these olefins to the extent that they could be determined quantitatively.

TABLE I

R_F AND $\lambda_{max.}$ VALUES FOR DIFFERENT COMPOUNDS AFTER METHOXYMERCURATION

Compound	R_F value	$\lambda_{max.}$ of coloured complex/nm
Oct-2-ene	0.81	540
Non-1-ene	0.87	540
2,2,4-Trimethylpent-2-ene ..	0.83	—
Cyclohexene	0.80	—
Styrene	0.78	—
Oleic acid	0.79	—
Mercury(II) acetate	0.35	525
Hexa-1,5-diene	0.04	560
Octa-1,7-diene	0.06	560

Two non-conjugated dienes, 1,7-octadiene and 1,5-hexadiene, were used to prepare the synthetic mixtures with different monoolefins, oleic acid and a conjugated diene. The results of analyses of these mixtures are given in Tables II and III. The contents of non-conjugated dienes found agree fairly well with the amounts taken. With 1,5-hexadiene, however, the error was 4.52–5%, possibly owing to evaporation losses during handling of the sample as it has a low boiling-point (59 °C).

TABLE II

DETERMINATION OF NON-CONJUGATED DIENES IN THE PRESENCE OF MONOOLEFINS

No. of mixture	Components of mixture	Concentration of olefins in the mixture, % m/V	Amount of the diene in the analysed sample/g	Amount of the diene found*/ g	Error, %	Standard deviation
1	Octa-1,7-diene ..	.. 8.599	0.085 99	0.083 67	2.69	±0.182
	Non-1-ene ..	.. 15.60				
2	Octa-1,7-diene ..	.. 17.385	0.173 85	0.170 59	1.66	±0.057
	Oct-2-ene ..	.. 14.237				
3	Octa-1,7-diene ..	.. 40.264	0.402 64	0.398 68	0.98	±1.68
	Styrene ..	.. 42.13				
4	Octa-1,7-diene ..	.. 3.732	0.037 32	0.037 6	1.28	±0.158
	Oleic acid ..	.. 13.437				
5	Hexa-1,5-diene..	.. 3.28	0.032 8	0.031 24	4.75	±0.225
	2,2,4-Trimethylpent-2-ene	.. 7.126				
6	Hexa-1,5-diene..	.. 3.006	0.030 06	0.028 63	4.42	+0.110
	Cyclohexene ..	.. 12.879				
7	Hexa-1,5-diene..	.. 4.412	0.044 12	0.041 89	5.05	±0.601
	Non-1-ene ..	.. 8.647				

* Mean value of four determinations.

2,3-Dimethyl-1,3-butadiene was taken as the conjugated diene and synthetic mixtures were prepared by mixing it with 1,7-octadiene in different proportions and then analysed (Table III). This conjugated diene reacted quantitatively with maleic anhydride and the Diels - Alder adduct resulting after methoxymercuration gave a very light-coloured spot (R_F 0.79) in the mono-adduct region. Attempts to determine simultaneously the monoolefin content from the same experiments did not give encouraging results.*

TABLE III

DETERMINATION OF NON-CONJUGATED DIENES IN THE PRESENCE OF CONJUGATED DIENES

No. of mixture	Components of mixture	Concentration of olefin in the mixture, % m/V	Amount of non-conjugated diene in the analysed sample/g	Amount of non-conjugated diene found*/g	Error, %	Standard deviation
1	Octa-1,7-diene ..	.. 68.858	0.338 3	0.325	3.96	±0.016
	2,3-Dimethyl-buta-1,3-diene	.. 3.27				
2	Octa-1,7-diene ..	.. 59.56	0.616 4	0.592	3.95	±0.024
	2,3-Dimethyl-buta-1,3-diene	.. 12.89				
3	Octa-1,7-diene ..	.. 39.958	0.399 9	0.387 3	3.1	±0.007
	2,3-Dimethyl-buta-1,3-diene	.. 32.458				

* Mean value of four determinations.

The authors are grateful to Dr. G. C. Joshi for his valuable comments.

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* In a control experiment, a known amount of monoolefin adduct was spotted on a silica gel plate. On extraction, without eluting the spot, the olefin could be determined quantitatively. However, when the spot was eluted on the plate and then extracted, the intensity of colour developed with diphenylcarbazone was about 4% of that expected. Apparently, during elution the adduct was demercurated.

Communication

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

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

Determination of Cyclohexanone in Intravenous Solutions Stored in PVC Bags by Gas Chromatography

Keywords: *Cyclohexanone determination*; *intravenous solutions*; *PVC bags*; *gas chromatography*

In 1970, di-2-ethylhexyl phthalate (DEHP) was identified in tissues of patients who had previously received blood transfusions.[1] The blood had been stored in poly(vinyl chloride) (PVC) bags plasticised with DEHP. Since then, much research has been carried out on the determination of phthalates (*e.g.*, refs. 2 and 3).

During our studies of DEHP in intravenous solutions stored in PVC bags, an unknown peak was observed on the total ion current trace of the gas chromatograph - mass spectrometer. Electron impact mass spectra (70 and 20 eV) indicated a compound of low relative molecular mass ($m/e = 98$) with the base peak at $m/e = 55$. On the basis of its fragmentation pattern, the compound was suspected to be cyclohexanone.[4] Its identity was verified by comparing the retention time and fragmentation with those of an authentic sample. The intravenous solutions investigated are listed in Table I.

Each PVC bag was enclosed in a diffusion-tight outer bag (a–f). A sample of the air surrounding the inner bag was analysed by using gas chromatography - mass spectrometry. When the instrument was focused on the base peak of cyclohexanone, a peak with a retention time identical with that of cyclohexanone was found. Headspace analyses of the plastic material of the PVC bags also gave a small peak with the same retention time.

Experimental

Apparatus

The identification and headspace analyses were performed on an LKB 2091 gas chromatograph - mass spectrometer equipped with a mass marker.

Quantitative determinations were performed on a Varian 1800 gas chromatograph equipped with a flame-ionisation detector. Columns (1.5 m × 2 mm i.d.) packed with 10% SP-1000 on Supelcoport (80–100 mesh) were used. The injector and the detector were both maintained at 160 °C. The column temperature was 90 °C and the nitrogen flow-rate was 30 ml min^{-1}.

Procedures

Determination of cyclohexanone in the solutions

Sodium hydrogen carbonate (0.5 g) and a solution of internal standard (1.0 ml of a 100 mg l^{-1} solution of hexan-1-ol in methanol) were added to 250 ml of the intravenous solutions (bags a–f). After mixing, the sample was extracted with 1.5 ml of chloroform and the extract was injected into the gas chromatograph. For the ACD and CPD solutions (bags g–h), 50 ml of the solution and 1.0 g of sodium hydrogen carbonate were used.

Calibration graphs for each sample were prepared from standard preparations. When blanks were analysed for contaminants, no trace of cyclohexanone was found. Retention times were 155 s for cyclohexanone and 190 s for the internal standard.

Headspace analyses of the PVC bags

The magnetic field was focused and locked on the base peak of cyclohexanone ($m/e = 55$). A

10-g amount of the PVC bag was introduced into a headspace vessel (25 ml) and sealed with a septum. After heating the vessel at 120 °C for 1 h, 1.0 ml was injected into the gas chromatograph - mass spectrometer.

Results and Discussion

Duplicate analyses were run from each solution in bags a–f, the differences between the parallel runs being 0–0.8 mg l^{-1} (Table I). The contents of cyclohexanone differed considerably also within the same batches (a–b and c–d). Recent productions contained the smallest amounts of cyclohexanone (g–h). Storage time and production conditions may contribute to the differences observed.

TABLE I
CYCLOHEXANONE CONTENTS OF INTRAVENOUS SOLUTIONS

Solution*	Age/ years	Cyclohexanone content/mg l^{-1}
(a) 500 ml of saline solution	2½	2.9,2.9
(b) 500 ml of saline solution	2½	8.0,8.8
(c) 500 ml of saline solution	3½	4.8,4.8
(d) 500 ml of saline solution	3½	7.2,6.7
(e) 500 ml of 5% glucose	3½	6.2,6.7
(f) 500 ml of Ringer solution	2	15.5,15.9
(g) 76.5 ml of ACD solution	1½	<1
(h) 63 ml of CPD solution	½	<1

* Samples a and b and samples c and d had the same batch numbers.

Samples a–f were taken from PVC bags supplied with printed declarations. Headspace analyses of the printing colour obtained from the producer did not reveal any cyclohexanone. This possible source of contamination being ruled out, it is suggested that use of cyclohexanone as a solvent during the production of the PVC bags may account for the reported findings.

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Received *August 19th*, 1977

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Book Reviews

PRACTICAL CLINICAL BIOCHEMISTRY. Fifth Edition. VOLUME 2. HORMONES, VITAMINS AND POISONS. By HAROLD VARLEY, ALAN H. GOWENLOCK and MAURICE BELL. Pp. xii + 402. London: William Heinemann Medical Books Ltd. 1976. Price £8.

Varley's Clinical Biochemistry has been a much used book of methods in almost all clinical laboratories in most English-speaking countries. This fifth, two-volume edition, revised by Varley and two collaborators, will be as welcome as previous editions.

When that famous predecessor of Varley's book, Peters and van Slyke's "Quantitative Clinical Chemistry, I, Methods," became out of print, most clinical chemists considered that a successor could not be achieved because of the explosive advances made. The five editions of Varley's book have shown how wrong this prediction was, although the exhaustive critical evaluation of methods put forward by Peters and van Slyke could not be continued without multiplying many-fold the size of the book. Volume II has preceded Volume I and includes a list of the contents of the latter.

The various methods of investigating thyroid disease are critically evaluated, although it is surprising that the automated method of measuring protein-bound iodine is presented in detail when medication by iodine-containing drugs, X-ray contrast media and oral contraceptives so frequently interfere with the determination. The use of thyrotrophin-releasing hormone is well described but it is surprising to find that the long-acting thyroid stimulator has not been given its up-to-date name of thyroid-stimulating immunoglobulin. The radioimmunoassay and competitive protein-binding methods are not described in detail because of the variety of kits available, but their essential features are compared and contrasted for the benefit of non-specialist laboratories. Other endocrinological techniques, especially those concerned with steroids, are meticulously described, each being provided with an account of the scientific and clinical basis of the need for the analysis. There usually follows a section on so-called normal values and interpretation of results. However, some of the methods, *e.g.*, the colorimetric determination of pregnanediol, might well have been omitted. Nevertheless, there are useful critical discussions of the tests appropriate to the clinical circumstances, together with descriptions of kinetic methods of investigating patients with various possible endocrinological abnormalities. The investigation of hirsuitism, infertility and virilism are briefly but well described. The account of synthetic hypothalamic hormones or their analogues, in pin-pointing the sites of abnormalities in pathways of endocrine activity, might well have been expanded.

The 42-page section on vitamins is useful but a possible indication of its importance in modern clinical biochemistry is that out of about 100 references only 35 are dated 1965 or later.

The 104-page section on drugs and poisons is especially valuable in providing information on many of the poisons and drugs often taken in overdose. However, many of the tests described can be applied after extensive separation procedures rather than after the inadequate extraction described, mainly on account of polypharmacy in modern prescribing. The Broughton method of determining barbiturates was of great importance when first developed but it is inappropriate with the availability of GLC, TLC and HPLC techniques. Not everybody would describe the colorimetric determination of lead as a well established *reference* method.

The volume is a mine of information with its list of references ranging from 1940 to 1975 and, despite criticisms, will rightly be used in many clinical biochemical laboratories.

C. H. GRAY

TREATISE ON ANALYTICAL CHEMISTRY. Part III. ANALYTICAL CHEMISTRY IN INDUSTRY. Volume 3. Edited by I. M. KOLTHOFF, PHILIP J. ELVING and FRED H. STROSS. Pp. xxiv + 598. New York, London, Sydney and Toronto: John Wiley. 1976. Price £27.70; $46.95.

Parts I and II of this comprehensive three-part treatise on analytical chemistry deal with *Theory and Practice*, and *Analytical Chemistry of Inorganic and Organic Compounds*, respectively.

Like the previous parts, some volumes of which have been reviewed in *The Analyst* (*q.v.*), Part III, *Analytical Chemistry in Industry*, contains several independent volumes, and this one (Volume 3) has three main sub-sections: (*C*) *Standards and Specifications*, (*D*) *Physical Testing Methods for*

the Characterization of Materials and (*D*-1) *Thermal and Chemical Testing*. Thus it will be appreciated that the book is only one of a series, all of which are planned to cover, *in toto*, a very wide field of analytical chemistry and its associated disciplines.

The Editors and, indeed, the individual authors are widely known throughout the world whereever the various aspects of analytical chemistry are taught or applied; a favourable point to emphasise by any reviewer who wishes to do justice to his assignment.

On this occasion, as with the earlier published volumes, the authors (this Volume has 15) were specially chosen for their acclaimed expertise in at least one of the individual fields, and the Editors' selection, aimed at providing a balanced industrial and academic approach, has been commendably achieved.

To provide a better appreciation of the coverage of this Volume, these are the title headings which appear under the three named sub-sections:

(*C*). *Standard Materials for Analysis and Testing, Standard Methods of Chemical Analysis and Standard-setting Organizations in the United States, Standard Methods of Test and Standard-setting Organizations in Europe, Statistical Methods in Analytical Chemistry*, and *Development and Utilization of a Quality Control Program.*

(*D*). *Rationale for Methods of Physical Testing, Introduction to Mechanical Testing*, and *Measurement in Bulk.*

(*D*-1). *Chemical Resistance of Nonmetallic Materials*, and *Thermal Aging and Oxidation with Emphasis on Polymers.*

To anyone engaged, or likely to be involved, in any detailed aspect of the multifarious subjects covered, the book will, no doubt, have a special appeal. For the busy industrial analyst, however, who has to delegate much of the work described to others who are usually more competent than himself in specific areas (in mechanical testing, to quote one possible example), this volume is unlikely to have the same appeal as the others in the series that I have seen. W. T. ELWELL

ELECTROCHEMICAL STRIPPING ANALYSIS. Edited by FRANTIŠEK VYDRA, KAREL ŠTULÍK and EVA JULÁKOVÁ. Translation Editor: Julian Tyson. *Ellis Horwood Series in Analytical Chemistry*. Pp. 283. Chichester: Ellis Horwood. Distributed by John Wiley, New York, London, Sydney and Toronto. 1976. Price £17.50; $33.25.

First impressions are always important, and one's first impression of this book is very favourable. It is very well presented, clearly printed on good paper and well bound. On beginning to read, the favourable impression is not lost. Dr. Tyson has made a very good job of the translation, and although there are a few places where the English is redolent of transliteration rather than translation, it would require an even more pedantic reviewer to take exception to them.

The authors begin with a survey of the theory of stripping analysis, and manage to cover the field in admirable detail in the first 50 pages or so. There is then a useful section on the properties of amalgams, where they have collected together a great deal of information from a wealth of different sources, followed by a section on the properties of films formed on mercury and solid electrodes.

Chapter 3, on the stripping process and monitoring methods, was felt to be rather a disappointment. As with so many Czech authors, the treatment of methods other than classical d.c. polarography is sketchy in the extreme, and gives the impression that the other techniques are of recent introduction. After 24 pages on "linear sweep polarography," which itself has a different connotation in the UK from the classical instrument and technique intended by the authors, coulometry receives 10 pages and sinusoidal a.c. polarography receives 6 pages, with square-wave polarography mentioned *en passant;* pulse polarography, the great hope of Western analytical electrochemists, is dismissed in less than half a page as being potentially useful, with the implication that it is fairly new and still requires a great deal of work to provide useful instruments.

Chapter 4, on apparatus, has a very good section on electrodes of many types, with enough information to enable the reader actually to use them; stationary mercury-drop and film electrodes, solid metal electrodes and graphite and carbon paste electrodes are all dealt with well. The section on measuring apparatus is less well covered, being devoted to very sketchy descriptions of various operational amplifier assemblies which would be of no help to an electronic neophyte. The final section on choosing actual analytical parameters is very well done, however, and serves

to underline the fact that the authors are electrochemists first and instrument technicians second.

The last 90 pages of the book are devoted to determination of the various elements, and finally examples of practical procedures are given.

The section on the elements includes, as is so regrettably inevitable, all of the usual paragraphs on the elements (alkali metals, alkaline earth metals, etc.), which no practical analyst would ever determine by stripping analysis, but does give good coverage of the metals for which it is really useful, backed up in some instances with tables collating references, electrodes, electrolytes and so on.

The book concludes with about 10 actual analytical applications of the method; it is perhaps noteworthy that in spite of the space given in the remainder of the book to electrodes other than mercury, only one of the practical examples uses an electrode that is not either a mercury drop or a mercury film.

R. C. ROONEY

PYROLYSIS–GAS CHROMATOGRAPHY. By R. W. MAY, E. F. PEARSON and D. SCOTHERN. *Analytical Sciences Monograph No.* 3. Pp. viii + 109. London: The Chemical Society. 1977. Price £7.20; $15; CS Members, £5.50.

This is the third volume in the Chemical Society Analytical Sciences Monograph series and discusses pyrolysis - gas chromatography (Py - GC) as an analytical technique. The book consists of five chapters which develop the theme of Py - GC from basic gas chromatography (46 refs.) through pyrolysis methods (121 refs.) and the application of the technique in analysis (145 refs.) to the methods available for fragment identification (38 refs.) and the problems of standardisation (21 refs.).

In these chapters the authors of this well produced but slim monograph have set out to present the available knowledge concerning Py - GC in a form suitable to practising analysts with the aims of aiding the choice of method and the avoidance of common pitfalls. The authors have largely achieved their aims and they have quoted or referenced much of interest, although only 15 or so of these sources appeared later than 1972. Inevitably, however, this approach has led to some re-statement of well understood principles in addition to necessitating brief treatment of important areas of current interest. An example of the former occurs in Chapter 1, which presents a familiar account of gas chromatography obviously aimed at the novice. That only one reference in this section is later than 1970 confirms this view. Examples of the latter are the treatment of biomedical applications, Py - GC - MS studies and structural or mechanistic inferences. Nevertheless, references are quoted to direct interested readers further.

Although not strictly within the compass of this monograph, the omission of direct pyrolysis - mass spectrometry, a current and developing technique, which yields additional information on less volatile pyrolysis fragments and also facilitates data handling, is to be regretted.

The authors have taken great care to stress the requirements for the standardisation and differentiation of pyrograms and they have devoted the final chapter to this topic. A valuable feature here is the inclusion of recommended conditions to produce a standard system and the provision of a library of 43 standard pyrograms derived from 29 plastics and 14 paint polymers. The library is indexed on a three major peak basis and the identities of many of the fragments are given.

Despite the lack of indexes, this volume forms a useful introduction to Py - GC and should be consulted by anyone contemplating the application of this technique.

W. J. IRWIN

CHROMATOGRAPHY OF STEROIDS. By E. HEFTMANN. *Journal of Chromatography Library, Volume* 8. Pp. xiv + 203. Amsterdam, Oxford and New York: Elsevier. 1976. Price Dfl90; $34.75.

One third of this monograph consists of a bibliography of 1 200 articles on steroid chromatography accumulated by the author over the period 1964–75. Since much of this literature has already been discussed in numerous reviews and books it would be of limited value if it were not for the fact that Heftmann, a pioneer in this field, has used his experience to select and organise this information in a single book.

After a brief introduction, the general techniques of liquid column chromatography, paper and thin-layer chromatography and gas chromatography are succinctly discussed in individual chapters dealing with the usage of currently available materials and instrumentation. The next chapter summarises attempted correlations between chromatographic mobility and steroid structure.

Individual steroid classes are then taken in turn and their liquid, thin-layer and gas - liquid chromatographic behaviour is reviewed in detail in chapters entitled sterols, bile acids, estrogens, androstanes, pregnanes, corticosteroids, etc. This section, Chapters 6–12, is probably the most useful part of the monograph. It will, for example, be of considerable value to a variety of research workers who, though not chromatographic experts, are nevertheless faced with an analytical problem related to such physiologically important compounds.

The author claims that the monograph is designed mainly as a laboratory handbook which "permits the reader to select the appropriate procedure which is often described in detail." It is hardly surprising that in a book of this size, covering such a wide range of compounds and techniques, that this claim is somewhat over-optimistic. The main value of the monograph will be for reference and an easy access to the relevant literature.

Since the monograph covers the period 1964–75 it is perhaps inevitable that there is some imbalance concerning currently used techniques. In this respect it is unfortunate that the enormous impact which HPLC is going to have on steroid analysis in the next decade is only briefly discussed. Nevertheless, the extensive chromatographic data reviewed should provide guidance in selecting suitable HPLC systems for a particular analysis.

The book is beautifully presented and contains few typographical errors. It is, however, somewhat expensive and while I would recommend it for a library as a reference book I have doubts about its value as an up-to-date laboratory handbook for the chromatography of steroids.

G. N. SMITH

ISOTACHOPHORESIS. THEORY, INSTRUMENTATION AND APPLICATIONS. By FRANS M. EVERAERTS, JO L. BECKERS AND THEO P. E. M. VERHEGGEN. *Journal of Chromatography Library, Volume* 6. Pp. xiv + 418. Amsterdam, Oxford and New York: Elsevier. 1976. Price Dfl160; $61.50.

Isotachophoresis, earlier called displacement electrophoresis, is an electrophoretic method carried out in a capillary tube, filled with a fast-moving ion and a buffer counter ion, connected with an electrode at one end supplying the counter ion and at the other with an electrode supplying a slow-moving ion. The sample to be analysed is introduced between the fast and slow ions.

As the sample ions move down the tube they separate in order of their net mobility with a more or less sharp boundary between each zone, which contains only one ion and the counter ion.

This is the first book on the subject and it will be essential for the serious student in spite of its price and the fact that the theoretical section has a mathematical bias and an absence of explanations which would have been of value to the uninitiated. Thus, there is no discussion of hydration of ions, but there is of their entropy. No adequate discussion is given of electroendosmosis and ways of reducing it. It is not pointed out that the concentration of ions in each succeeding zone decreases because the velocity of the counter ion has increased as it enters the raised potential gradient of the succeeding ion. There is inadequate discussion of the sharpness of the fronts between zones, although this of prime importance. No theory of the temperature front is given, although it explains the inferiority of temperature measurement as a detector. No equation is given to determine the pH difference between succeeding zones, although a long and difficult discussion describes the method used in a computer program which is also given to determine pH.

The book has obviously been made up from a series of papers published by the authors and the lacunae between them has been filled sketchily or not at all.

In contrast, the instrumental part is excellent. It is perhaps slightly long as a history of their apparatus is given. The section on detectors merits special praise.

Everaerts and his team, virtually alone, have brought the state of the art of this method of analysis in about 10 years to be comparable with that of gas chromatography. It is now possible to analyse a mixture, in a reasonable case, with a dozen components in less than 1 min with an error of about $\pm 2\%$ and with a minimum amount of one substance of 100 pmol. Various advances have contributed to this: resistance and ultraviolet detectors, special treatment of detector electrodes to increase overvoltage and additives to the solution to reduce electroendosmosis. As a result, it has been feasible to reduce the bore of the capillary tube, with an increase in the permissible potential gradient, now about 2 kV cm^{-1}, giving smaller amounts and sharper fronts which can still be detected. Even uni- and polyvalent ions can now be distinguished.

The separation of proteins has lagged badly, but in due course, displacement electrophoresis may well open a new era.

The reviewer wishes to air a grouse that the name displacement electrophoresis, which he used, has been dropped. Isotachophoresis (all moving at the same speed) is a true description only of a perfect apparatus in the neighbourhood of the detector—it tells nothing of the process of separation and it is never true of the whole apparatus. In contrast, displacement electrophoresis correctly describes what is happening during the whole process of separation and in sharpening the fronts against diffusion and defects such as temperature differences, electroendosmosis or curvature or irregularity of the tube. It is responsible for bringing dilute traces in the sample to wafer-like zones of a concentration similar to the concentrations of neighbouring zones. It is the displacement of an ion by a change of potential gradient that keeps ions of one kind together. The name also shows the kinship with displacement chromatography named by Tiselius, which has many similar characteristics.

A. J. P. MARTIN

THERMAL ANALYSIS OF MINERALS. By DUMITRU N. TODOR. Pp. 256. Tunbridge Wells: Abacus Press. 1976. Price £12.50.

This book is an English translation of the 1972 Rumanian publication "Analiza Termică a Mineralelor." As a contribution to the field of thermal analysis of minerals, it is somewhat disappointing, especially as no attempt has been made to update the references to take account of thermal analysis developments since 1972. The style of translation is not always good, *e.g.*, "Enthalpy is a magnitude of state . . . . . " (p. 11) is hardly meaningful; "nicely crystallised" (p. 172) would have been better represented as "well crystallised." Numerous spelling errors occur throughout the text and some, such as "gloserite" (p. 125) for "glaserite," might cause confusion. References to the first person in the experimental work give an untidy impression and lack of indexing of the minerals described makes the book a less convenient source of reference. However, the numerous thermal analysis curves shown are clearly illustrated and of value to the reader.

Explanations in the text are generally reasonably clear, but important omissions occur. Although the techniques of thermogravimetry (TG), differential thermal analysis (DTA), differential thermogravimetry (DTG) and thermal derivatography are fairly well explained in Chapter 1, other important thermal analysis methods such as differential scanning calorimetry (DSC) and evolved gas analysis (EGA) are ignored. Types of sample holders for atmosphere control are not discussed in Chapter 2 on experimental factors. There is no exemplification in Chapter 3 of recommendations for the communication of analytical data (p. 73) to facilitate understanding.

The classifications of zeolitic and colloidal water in Chapter 4 (p. 91) as types of free water are, to say the least, questionable.

The thermal analysis of minerals in Chapter 5 is concisely described, but is rendered less valuable by the following points. The scheme for the polymorphous transformation of quartz (p. 102) includes tridymite, which has been shown not to be a pure silica form, but a solid solution of mineralisers. In gypsum dehydration (p. 127), the formation of soluble anhydrite by water loss from the hemihydrate is not mentioned. For calcium carbonate (p. 159) the important metastable form vaterite is ignored. With silicates, although the reader is told (p. 188) that they can be classified in several ways, no references to such classifications are given, nor are feldspars and molecular sieves mentioned. It would have been more correct to describe clays as being based on the $Si_4O_{10}^{4-}$ instead of the $Si_2O_5^{2-}$ anion, so as to indicate the sheet-like nature of the silicate groupings therein. No literature references are given either for methods of separating clays for analysis (p. 212) or for detailed procedures (Chapter 6) to carry out thermal analysis methods for investigating complex rocks.

Provided that the above points are borne in mind, the book has some value for students and others wishing to acquaint themselves with TG, DTA and DTG of minerals, but the relatively high price might tend to confine its use largely to being a reference text in libraries.

JOHN BENSTED

Determination of Reducing Sugars in Honey, Marmalades and Fruit Juices Using a Copper Ion-Selective Electrode

A potentiometric method for the determination of reducing sugars is described. The sample is treated with Stanley - Benedict reagent and the amount of unreacted copper(II) is determined with a copper-selective electrode by use of the standard addition method; it is then related directly to the amount of reducing sugar. Amounts of glucose in the range 0.25–2 mg have been determined with an average error of about 2%. The method has been applied to the analysis of honey, marmalades and fruit juices. The analytical recovery of glucose was 99–106%. Comparison with an official method gave satisfactory results.

Keywords: *Copper ion-selective electrode*; *standard addition potentiometry*; *reducing sugars determination*; *honey and fruit products analysis*

D. S. PAPASTATHOPOULOS, D. P. NIKOLELIS and T. P. HADJIIOANNOU

Laboratory of Analytical Chemistry, University of Athens, Athens, Greece.

Analyst, 1977, **102**, 852–857.

Determination of Residues of Organophosphorus Pesticides in Fruits and Vegetables

Report by the Panel on Determination of Residues of Certain Organophosphorus Pesticides in Fruits and Vegetables

Keywords: *Organophosphorus pesticide residues*; *fruits*; *vegetables*; *gas - liquid chromatography*

COMMITTEE FOR ANALYTICAL METHODS FOR RESIDUES OF PESTICIDES AND VETERINARY PRODUCTS IN FOODSTUFFS (N. A. SMART, SECRETARY)

Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory, Hatching Green, Harpenden, Hertfordshire, AL5 2BD.

Analyst, 1977, **102**, 858–868.

Determination of Sulfanitran in Animal Feeds and Pre-mixes

Report prepared by the Medicinal Additives in Animal Feeds Sub-Commitee A.

Keywords: *Sulfanitran determination*; *animal feeds*; *spectrophotometry*

ANALYTICAL METHODS COMMITTEE

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

Analyst, 1977, **102**, 869–872.

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Improved Method for the Determination of Pyrimethamine in Poultry and Rabbit Feeding Stuffs by Gas - liquid Chromatography

Short Paper

Keywords: *Pyrimethamine determination*; *animal feeding stuffs*; *gas - liquid chromatography*

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Analyst, 1977, **102**, 873–876.

Determination of Iodine Using a Kinetic Method with an Iodide Ion-selective Electrode

Short Paper

Keywords: *Iodine determination*; *iodide ion-selective electrode*; *water analysis*; *kinetic method*

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Analyst, 1977, **102**, 876–878.

Determination of Diolefins in Olefinic Cracked Hydrocarbon Products: Determination of Model Compounds

Short Paper

Keywords: *Diolefin determination*; *cracked hydrocarbons*; *methoxymercuration*; *spectrophotometry*; *thin-layer chromatography*

M. P. SAXENA and K. K. BHATTACHARYYA
Indian Institute of Petroleum, Dehradun, India.

Analyst, 1977, **102**, 878–881.

Determination of Cyclohexanone in Intravenous Solutions Stored in PVC Bags by Gas Chromatography

Communication

Keywords: *Cyclohexanone determination*; *intravenous solutions*; *PVC bags*; *gas chromatography*

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Analyst, 1977, **102**, 882–883.

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