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Summaries of Papers in this Issue

Spectrophotometric Determination of Aluminium in Alloys and Ores. Part 1. Removal of Interfering Metals as Their 2-Isopropylquinolin-8-ol Chelates by Precipitation and Extraction

A simple and rapid method for determining aluminium in permanent magnet alloy, iron and manganese ores and nimonic 90 alloy is described. The sample is dissolved in acid and the aluminium is separated from the interfering metals such as iron, cobalt, nickel, chromium, copper, manganese and titanium by extracting these into chloroform as their 2-isopropylquinolin-8-ol chelates. The aluminium is left behind and is extracted into chloroform as its quinolin-8-ol chelate and determined spectrophotometrically. For aluminium contents ranging from about 7 to 1.4% the method uses a sample mass of between 500 and 2000 μg present in the aliquot taken and can therefore be considered as a useful microchemical technique.

Keywords: *Aluminium determination*; *spectrophotometry*; *2-isopropylquinolin-8-ol separation*

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Analyst, 1981, **106**, 1137–1144.

Spectrophotometric Determination of Aluminium in Alloys and Ores. Part 2. Stripping Aluminium after Chelation of Other Metals with 2-Isopropylquinolin-8-ol in Butan-1-ol

In Part 1, aluminium was separated after extracting the other metals present, as their 2-isopropylquinolin-8-ol chelates, from aqueous solutions. In this paper a simpler and more efficient method of separation of aluminium is described. Instead of precipitating the interfering metals as their 2-isopropylquinolin-8-ol chelates and then extracting them into chloroform, they are prepared directly in butan-1-ol. Chelation of aluminium with the 2-isopropyl compound does not occur in butan-1-ol medium. The butanolic solution containing unchelated aluminium and the chelates of other metals is mixed with chloroform and then extracted with alkaline tartrate-buffered solution. In this way the uncomplexed aluminium is transferred to the aqueous phase, from which it is extracted as its quinolin-8-ol complex and determined spectrophotometrically. The technique has been applied to the determination of aluminium in complex matrices such as permanent magnet and nimonic 90 alloys.

Keywords: *Chelation of metals*; *2-isopropylquinolin-8-ol*; *butan-1-ol*; *solvent extraction*

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Analyst, 1981, **106**, 1145–1149.

Solvent Extraction of the Thiocyanate Mixed-ligand Complexes of Iron(III) with Various Hydroxyamidines and Spectrophotometric Determination of Iron(III) in Various Biochemical and Biological Samples

The reaction in benzene of 11 newly synthesised *N*-hydroxy-*NN'*-diarylbenzamidines (HOA) with iron(III) in the presence of thiocyanate has been investigated spectrophotometrically. The study revealed the formation of a 1:2:1 iron(III) - thiocyanate - HOA mixed complex in acidic media (0.2–0.8 M hydrochloric acid). On the basis of this sensitive colour reaction, a simple, rapid, selective and highly reproducible method for the extractive - spectrophotometric determination of microgram amounts of iron(III) in various biochemical and biological samples has been developed. The molar absorptivities of the systems are found to be between 1.1 and 1.35 $\times 10^4$ l mol^{-1} cm^{-1} in the wavelength range 460–470 nm. The method is free of interference from most of the common metal ions and commonly used sequestering agents. The effects of experimental variables on the procedure are discussed.

Keywords: *Solvent extraction*; *spectrophotometry*; *iron*(III) - *thiocyanate complex*; *hydroxyamidines*

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Analyst, 1981, **106**, 1150–1156.

Utility of π-Acceptors in Charge-transfer Complexation of Alkaloids: Chloranilic Acid as a Spectrophotometric Titrant in Non-aqueous Media

A spectrophotometric titration method is described for the determination of some alkaloids and their dosage forms using 0.005 M chloranilic acid solution in 1,4-dioxan as the titrant. The end-point is determined by measuring the change in absorbance of the sample at 535 nm. Quantitative recoveries with good reproducibility are reported for atropine, emetine, reserpine, strychnine, yohimbine and four dosage forms. The least-squares method for the end-point location in the spectrophotometric titration is also proposed.

Keywords: *Spectrophotometric titration*; *alkaloid determination*; *chloranilic acid*; *charge-transfer complexation*

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Analyst, 1981, **106**, 1157–1162.

Spectrophotometric Determination of Piperazine via Charge-transfer Complexes

Iodine - piperazine or chloranil - piperazine charge-transfer complexes have been used for the sensitive assay of piperazine or its salts; these complexes exhibit intense absorption bands in the electronic spectrum. The molecular ratios of the reactants in the complexes have been established and the experimental conditions leading to maximum charge-transfer bands were also studied. The proposed procedures have been applied successfully to pure samples and drug formulations with good accuracy. The average recovery was 100.26–100.84% with piperazine - iodine and 99.33–100.33% with piperazine - chloranil charge-transfer complexes, with an average standard deviation for each method of 0.8–3.9%.

Keywords: *Piperazine and piperazine salt determination*; *spectrophotometry*; *charge-transfer complexes*; *drug formulations*

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Analyst, 1981, **106**, 1163–1167.

Simultaneous Determination of Eight Trace Elements in Human Skin by Instrumental Neutron-activation Analysis

Eight trace elements (scandium, chromium, iron, cobalt, zinc, selenium, rubidium and caesium) were determined simultaneously in human skin by instrumental neutron-activation analysis. The concentrations of all trace elements studied were higher in the abdominal epidermis than the dermis. Site specification was found to be essential.

Application of cluster analysis revealed that iron and zinc were the most similar trace elements in their distribution in the dermis relative to the others studied, and chromium in the epidermis was the most dissimilar.

Keywords: *Trace element determination*; *epidermis*; *dermis*; *cluster analysis*

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Analyst, 1981, **106**, 1168–1173.

Method for the Simultaneous Determination of Arsenic, Aluminium, Iron, Zinc, Chromium and Copper in Plant Tissue Without the Use of Perchloric Acid

A 0.5-g sample of plant tissue was digested with a mixture of concentrated nitric and sulphuric acids (2 + 1 *V*/*V*) and hydrogen peroxide. Arsenic was determined by the hydride generation method. Aluminium, iron, zinc, chromium and copper were determined by direct flame atomic-absorption spectrometry. The detection limits in dry plant material using 50 ml of aqueous solutions for analysis were 0.5 ng g^{-1} for arsenic and 0.1 $\mu g\ g^{-1}$ for aluminium, iron, zinc, chromium and copper. The relative standard deviations were 4, 6, 1, 11, 6 and 7%, respectively. All six metals were determined from the same aliquot with recoveries ranging from 93 to 118%. A study was made of the composition of the precipitate that settled out from the extracts. X-ray diffraction revealed the presence of α-aluminium oxide (corundum) and some quartz in the anti-bumping granules. α-Aluminium oxide was a source of contamination for the aluminium analysis.

Keywords: *Metal determination*; *plant tissue*; *atomic-absorption spectrometry*; *X-ray diffraction*; *acid digestion*

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Analyst, 1981, **106**, 1174–1178.

Some Observations on the Capabilities of Photoacoustic Fourier Transform Infrared Spectroscopy (PAFTIR)

The interfacing of a photoacoustic cell to a Fourier transform infrared spectrometer is described. The performance of the system with some powder and film samples has been evaluated and the differences between the photoacoustic spectra and corresponding transmission spectra are discussed. Data are presented that indicate the possibility of quantitative analysis employing the PAFTIR system.

Keywords: *Photoacoustic spectroscopy*; *infrared spectroscopy*; *Fourier transform infrared spectroscopy*; *interferometry*; *polymer characterisation*

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Analyst, 1981, **106**, 1179–1186.

Pattern Display for Characterisation of Trace Amounts of Odorants Discharged from Nine Odour Sources

The odorants discharged from nine odour sources were classified into eight compound groups and were analysed by a systematic gas-chromatographic technique. The characterisation of trace amounts of the odorants was carried out by using the values for new proposed units (pOU_m, pOU_a, logOU, OU_t and OU; all terms are dimensionless) based on the ratio of the detected concentration to the odour recognition threshold concentration. The graphical representation of these data is effective for rapid recognition of the whole state. A polar co-ordinate pattern display was also proposed for the explanation of the relationship between odour characteristics (odour quality and intensity) and chemical analysis data of the odorants responsible for each odour discharged from nine odour sources. The calculated pOU_m and pOU_a values of eight odorant groups were plotted on polar co-ordinate circular odour charts. These charts illustrated a characteristic pattern and it was found that the shapes and sizes of each odour chart could characterise the quality and intensity of each odour from the nine odour sources. This was confirmed by investigating examples of processes or factories belonging to the nine odour sources.

Keywords: *Odorant characterisation*; *air analysis*; *gas chromatography*; *cold- and adsorption-trapping*; *pre-column concentration*

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Analyst, 1981, **106**, 1187–1202.

Separation and Identification of Aminocarboxylic Acid Sequestrants by High-performance Liquid Chromatography

A simple method has been developed for the separation by high-performance liquid chromatography of some common aminocarboxylic acid sequestrants as their copper complexes. The copper complex is formed *in situ* by using a copper salt solution as the eluate. Detection using visible light at 760 nm eliminates interference from ultraviolet absorbing compounds often present in mixtures containing sequestrants.

Keywords: *High-performance liquid chromatography*; *aminocarboxylic acids*; *sequestrants*; *copper complexes*

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Analyst, 1981, **106**, 1203–1207.

Determination of Pyrimethamine in Animal Feeds

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

Keywords: *Pyrimethamine determination*; *animal feeds*; *gas - liquid chromatography*

ANALYTICAL METHODS COMMITTEE

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Analyst, 1981, **106**, 1208–1209.

Determination of Tungsten in its Ores and Concentrates by Atomic-absorption Spectrometry

Short Paper

Keywords: *Tungsten determination*; *ores*; *atomic-absorption spectrometry*

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Analyst, 1981, **106**, 1210–1213.

Differential-pulse Polarographic Determination of Degradation Products of Cephalosporins: Comparison of the Degradation of Cephaloglycin in Neutral Solution with that of Cephalexin

Short Paper

Keywords: *Differential-pulse polarography*; *cephaloglycin*; *degradation*

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Analyst, 1981, **106**, 1213–1217.

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The Analyst

Spectrophotometric Determination of Aluminium in Alloys and Ores

Part 1.* Removal of Interfering Metals as Their 2-Isopropylquinolin-8-ol Chelates by Precipitation and Extraction

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A simple and rapid method for determining aluminium in permanent magnet alloy, iron and manganese ores and nimonic 90 alloy is described. The sample is dissolved in acid and the aluminium is separated from the interfering metals such as iron, cobalt, nickel, chromium, copper, manganese and titanium by extracting these into chloroform as their 2-isopropylquinolin-8-ol chelates. The aluminium is left behind and is extracted into chloroform as its quinolin-8-ol chelate and determined spectrophotometrically. For aluminium contents ranging from about 7 to 1.4% the method uses a sample mass of between 500 and 2000 μg present in the aliquot taken and can therefore be considered as a useful microchemical technique.

Keywords: *Aluminium determination*; *spectrophotometry*; *2-isopropylquinolin-8-ol separation*

Ever since Merrit and Walker[1] showed that 2-methylquinolin-8-ol chelates with many metals but not with aluminium, methods have been developed with a view to separating aluminium from other metals associated with it by the use of this reagent. However, the results obtained have not been satisfactory, especially when aluminium was a minor constituent. Pantony and Selfe (unpublished work) found that quantitative separation of aluminium using 2-methylquinolin-8-ol was possible only for binary alloys of aluminium with magnesium, nickel, copper or beryllium. They also found that despite varying extraction conditions the results obtained for aluminium in complex alloys and iron ore using 2-methylquinolin-8-ol separation were high. Their observations were supported by the method described by Hynek and Wrangell,[2] who used mercury cathode electrolysis in conjunction with 2-methylquinolin-8-ol to separate the interfering elements from aluminium in complex alloys. Although they obtained accurate results by this method, it appears to have no great advantage over existing methods.

According to Dagnall *et al.*,[3] aluminium is in fact extracted by 2-methylquinolin-8-ol into chloroform to a certain extent. They showed that the recovery of aluminium after preliminary extraction with 2-methylquinolin-8-ol was low. Hence there is a degree of uncertainty about the quantitative results obtained for aluminium using 2-methylquinolin-8-ol for the removal of interfering elements.

The use of 2-isopropylquinolin-8-ol for the separation of aluminium from interfering elements might be of advantage in two ways:

1. substitution of the 2-isopropyl group in place of 2-methyl will cause increased steric hindrance and therefore prevent the extraction of aluminium; and
2. the higher solubility ratios of the chelates of this alkyl homologue compared with those below it should ensure more complete removal of the interfering metals.

With this objective, previous investigators[4,5] synthesised 2-ethyl-, 2-isopropyl- and 2-*tert*-butylquinolin-8-ols. Although the synthesis of the reagents and their physico-chemical properties were studied, their analytical usefulness was not firmly established. Haba[4] used the 2-ethyl and 2-isopropyl homologues for separating aluminium in a complex alloy and

* For Part 2 of this series, see p. 1145.

found that the 2-isopropyl homologue gave satisfactory results for aluminium. Kazi,[5] however, found that the method described by Haba produced high aluminium figures. This indicated that Haba's method was not effective in removing the metals that interfere in the spectrophotometric determination of aluminium by quinolin-8-ol. It was therefore decided to investigate the best experimental conditions for the removal of interfering elements by 2-isopropylquinolin-8-ol prior to the determination of aluminium in different matrices.

Experimental

2-Isopropylquinolin-8-ol was synthesised by the direct alkylation of quinolin-8-ol by the appropriate lithium alkyl according to methods described previously.[5,6]

Preliminary Investigations

The wide pH range over which metal ions such as iron, copper, nickel, cobalt, manganese and zinc are reported[4,6] to be extracted into chloroform by 2-isopropylquinolin-8-ol, and the failure of this reagent to react with aluminium under any conditions, indicated the feasibility of separating aluminium from the other metals, when present. However, extraction of metals chelated by the 2-isopropyl compound was not rapid when the aqueous solution of the metal ion at a suitable pH was shaken with a solution of the reagent in an organic solvent such as chloroform. This has been explained elsewhere[6] in terms of kinetic factors involved in the extraction process. This difficulty was overcome by precipitating the metal chelates from the aqueous solution at a suitable pH and then extracting them into chloroform. In this method of extraction the number of precipitations and extractions required to remove the interfering metal ions depended on the total amount of metals present, the valence states of the metal ions, the concentration of the reagent and the time allowed for the precipitation of the metal chelates at the chosen pH.

Investigations on various metal ions either independently or in combinations were carried out to find the maximum amount of metals that could be efficiently separated so that the limitation of the technique to the determination of aluminium could be ascertained.

Efficiency of Removal of Interfering Metal Ions by 2-Isopropylquinolin-8-ol

Synthetic alloy solutions containing various amounts of iron, copper, nickel, cobalt and manganese were prepared from Specpure metals. A 5-ml fraction containing a total amount of the metal ions between about 4 and 6 mg was pipetted out into a 100-ml separating funnel. To this were added 10 ml of 1% *m/V* tartaric acid followed by 10 ml of 2-isopropylquinolin-8-ol (1% *m/V* in 0.2 M hydrochloric acid). The pH was adjusted to 9.5–10 with 1 M sodium hydroxide solution and the mixture was allowed to stand for 5 min. The precipitated chelates were extracted into 15 ml of chloroform and the organic phase was discarded. These operations were repeated on the aqueous phase but using only half the amount (5 ml) of the reagent for precipitation and 10 ml of chloroform for extraction each time, until the chloroform extract was colourless.

The aqueous phase was washed with 5 ml of chloroform and the washings were discarded. After adjusting the pH of the aqueous phase to 5 with glacial acetic acid, it was extracted with 5-ml portions of 1% *m/V* quinolin-8-ol in chloroform. The extracts were made up to 10 ml, dried with anhydrous sodium sulphate and the absorbance was measured at 400 nm, the wavelength suitable for the aluminium - quinolin-8-ol complex. A blank determination using all of the reagents (except the synthetic alloy solution) was conducted using the same procedure. The absorbance measured for the quinolin-8-ol extract obtained from the synthetic alloy (after 2-isopropylquinolin-8-ol extraction) was not significantly different from the reagent blank. This indicated the efficiency of removal of metals that would interfere in the determination of aluminium by quinolin-8-ol.

Separation and determination of aluminium were attempted by adding known amounts of aluminium to synthetic alloy mixtures. The results are presented in Table I.

Although the results in general were slightly low, they were considered reasonable for routine purposes. In synthetic alloy solution B the total mass of metals separated was more than in A and therefore required an increase in the number of extractions. Also in the synthetic alloy solution B the higher concentration of nickel and copper compared with A was considered to cause the inefficient extraction of iron(III), which chelates less readily

than the bivalent metals, accounting for an increase in the number of extractions required. This possibly could have incurred some loss of aluminium, resulting in a slightly low recovery. However, there was sufficient indication that by an appropriate choice of sample mass the method can be used advantageously for determining aluminium above 1% *m/m* levels in fairly complex alloys.

TABLE I

ALUMINIUM RECOVERED AT DIFFERENT LEVELS AFTER 2-ISOPROPYLQUINOLIN-8-OL SEPARATION OF IRON, COPPER, NICKEL, COBALT AND MANGANESE

Sample	Total mass of matrix metals in 5-ml aliquot/μg	Composition of matrix metals		Aluminium equivalent to the amount added, %	Aluminium, found, %
		Metal	Amount/μg		
Synthetic alloy A	4176.4	Iron	2792.5	0.239	0.226
		Copper	317.9		
		Nickel	291.0		
		Cobalt	500.0		
		Manganese	275.0		
Synthetic alloy B	5734	Iron	2234	0.105	0.093
		Copper	635	0.157	0.144
		Nickel	2355	0.209	0.187
		Cobalt	400	0.244	0.217
		Manganese	110		

Interferences Due to Chromium and Titanium

The fact that chromium is not extracted at room temperature by quinolin-8-ol and its 2-methyl, 2-ethyl and 2-isopropyl homologues was considered favourable for the separation of aluminium from chromium. However, in alloys rich in chromium (about 20% *m/m*), nickel (about 59%) and cobalt (about 15%) and low in titanium, iron and aluminium, the recovery of aluminium varied widely when separation of metals other than chromium and aluminium was carried out as described previously. It was noticed in these instances that a precipitate developed as a "veil" at the interface, resulting in unsatisfactory extraction conditions, especially during the extraction of aluminium by quinolin-8-ol. When chromium was expelled as chromyl chloride prior to the extraction of other matrix metals with the 2-isopropyl compound, hardly any "veil" was noticeable. As there was a possibility of losing aluminium by volatilisation when chromium was expelled as chromyl chloride, this method was not preferred. It was therefore decided to precipitate chromium together with other metals by 2-isopropylquinolin-8-ol from hot solution. Interference due to chromium in aluminium extraction could therefore be eliminated.

Although it would seem attractive to precipitate chromium together with other metal chelates of the 2-isopropyl compound from a hot tartrate-buffered solution in a single step, it was found that tartrate made the precipitation of chromium incomplete. This is in agreement with published information.[7,8]

However, the use of an acetate-buffered medium and a pH of 5 precipitated most of the chromium, which was then extracted into chloroform. A second precipitation was conducted in a hot tartrate-buffered medium at pH 10. At this pH, which was suitable for the precipitation of nickel, cobalt and manganese chelates of the 2-isopropyl compound, any residual chromium remaining in the solution was also precipitated.

Experiments conducted on synthetic alloy solutions containing chromium, nickel, cobalt, iron and titanium to examine the efficiency of removal of these metals showed that the blank was, on average, 0.02 absorbance unit higher than the blanks from the reagents. The aluminium equivalent to this absorbance was 0.08 μg ml^{-1} in the final quinolin-8-ol - chloroform extract. As the metal solutions were prepared from Specpure materials with an aluminium content of between 1 and 10 p.p.m. they could not be expected to make significant contributions to the higher absorbance noted for the synthetic alloy blank. Investigations showed that incomplete removal of titanium contributed to the higher absorbance noted for the synthetic alloy.

Influence of Titanium

In the absence of chromium, titanium at the 1% *m/m* level does not interfere. In the presence of chromium, titanium at the 50-μg level (equivalent to 2.5% *m/m* of the total metals) in the solution analysed was removed only up to 90%. However, if the quinolin-8-ol extraction of aluminium in the final solution was carried out at pH 11 trace amounts of titanium present were not extracted as indicated by the synthetic alloy blank not being significantly different from the blank obtained from the reagents. Experiments on the synthetic alloy with known amounts of added aluminium showed the method to be suitable for similar alloys containing 1–2% *m/m* of aluminium using a sample mass of 2 mg.

Determination of Aluminium in Alloys and Ores

Apparatus

Spectrophotometer.
Silica or glass cuvettes, path length 10 mm.
Separating funnels, 100 *ml, with PTFE keys.*
PTFE beakers, 50 ml.

Reagents

Doubly distilled water and analytical-reagent grade acids were used throughout.
Hydrochloric acid, sp. gr. 1.18.
Nitric acid, sp. gr. 1.42.
Perchloric acid, sp. gr. 1.54.
Sulphuric acid, sp. gr. 1.84.
Acetic acid, glacial.
Tartaric acid. 0.5 or 1% *m/V* as required.
2-Isopropylquinolin-8-ol. 2 or 4% *m/V* in 0.4 M hydrochloric acid, or solid reagent, as required.
Sodium hydroxide solution, 1 M. Prepared from Ultrar grade reagent.
Quinolin-8-ol. The commercially available reagent was steam distilled and crystallised from hexane and a 1% *m/V* solution in chloroform was prepared.
Chloroform. Analytical-reagent grade; freshly distilled if necessary.
pH indicator paper strips. Strips of good quality were used.

Sample mass

The sample mass was so chosen that an aliquot taken resulted in at least 1 μg ml^{-1} of aluminium in the final volume of the quinolin-8-ol chloroform extract. The maximum concentration of aluminium should preferably be not more than 2.4 μg ml^{-1}.

Aluminium in Permanent Magnet Alloy

Permanent magnet alloy [British Chemical Standard (BCS) 233], chosen as a typical alloy for the study, has the following composition: iron 51.15, nickel 11.22, cobalt 23.72, copper 5.09, titanium 0.79, aluminium 6.99 and manganese 0.235%.

Preparation of sample solution

A 0.1-g amount of carefully sampled alloy was dissolved in 10 ml of hydrochloric acid (sp. gr. 1.18). A 2-ml volume of nitric acid (sp. gr. 1.42) was added to complete the dissolution and the solution was taken to fumes with 10 ml of perchloric acid (sp. gr. 1.54). The solution was cooled and diluted with 100 ml of water and gently warmed to dissolve the salts. It was then treated with a further 5 ml of perchloric acid and made up to 1 l with water.

Extraction procedure

An aliquot representing about 500 μg of sample was pipetted into a 100-ml separating funnel and treated with 5 ml of 0.5% *m/V* tartaric acid solution. This was followed by the addition of 5 ml of a 2% *m/V* solution of 2-isopropylquinolin-8-ol in 0.4 M hydrochloric acid. The pH was adjusted to 10 (with the help of appropriate pH indicator paper strip) with

1 M sodium hydroxide solution and the mixture was shaken for 2 min. The precipitate was allowed to stand for 10 min and extracted into 15 ml of chloroform, the chloroform phase being discarded. The aqueous phase was treated with 5 ml of 2-isopropylquinolin-8-ol and mixed well. Precipitation and extraction were repeated as before using only 10 ml of chloroform for extraction. A third precipitation and extraction step was conducted under similar conditions using only half the amount of the 2-isopropyl reagent. The aqueous phase was washed with 5 ml of chloroform and the washings were rejected. The solution was adjusted to pH 5 with glacial acetic acid and extracted successively with 10- and 5-ml portions of 1% quinolin-8-ol in chloroform. The chloroform extracts were made up to 25 ml with chloroform and dried with anhydrous sodium sulphate. The absorbance of the extract was measured at 400 nm on a Hilger and Watts Uvispek spectrophotometer against chloroform. A blank run was conducted under the same conditions used for the sample.

A stock solution containing 1 g l^{-1} of aluminium was prepared by dissolving Specpure aluminium in hydrochloric acid, and from this a range of aluminium standards were prepared by extraction. The best straight line fit for the graph plotted of absorbance *versus* aluminium concentration was obtained with the help of a computer. Using the slope of the line the aluminium content in the sample was calculated after deducting the blank. The results are presented in Table II.

Alumina in Iron Ore

BCS 301 Lincolnshire iron ore was taken for this analysis and treated according to the following procedure.

Preparation of sample solution

Method 1. About 0.1 g of dried sample (dried at 105 °C) was accurately weighed into a platinum basin and covered with 2 ml of water. It was then treated with 5 ml of hydrochloric acid (sp. gr. 1.18), keeping the basin covered with a glass cover to prevent losses due to effervescence. When dissolution was complete the glass cover was removed and rinsed into the basin with the minimum possible amount of water. The solution was treated with 1 ml of hydrofluoric acid (40% *m*/*m*) and 5 ml of perchloric acid (sp. gr. 1.54) and evaporated to fumes. After fuming for 10 min it was cooled and treated with 2 ml of water and evaporated to fumes again. It was then cooled, diluted with 10 ml of water and gently heated to dissolve the salts. As there was no noticeable residue left at this stage the solution was made up to 100 ml with water after adding a further 2 ml of perchloric acid.

Method 2. In this method about 0.2 g of sample was weighed and dissolved in a mixture of 4 ml of water and 10 ml of hydrochloric acid (sp. gr. 1.18). After complete dissolution it was evaporated almost to dryness. The residue was covered with 5 ml of hydrochloric acid (sp. gr. 1.18) and 15 ml of water and gently boiled. After diluting with water to 50 ml, the solution was filtered through a 9-cm filter-paper (No. 40) into a 250-ml calibrated flask. The insoluble material was transferred and washed with 3% *V*/*V* hydrochloric acid. The washing was finally completed with water. The filter-paper was ashed in a platinum basin at 900 °C, the ash was covered with 1 ml of water and 0.5 ml of sulphuric acid (1 + 1), then treated with 5 ml of hydrofluoric acid and evaporated until fumes of sulphur trioxide began to be evolved vigorously. After fuming to dryness the residue was fused with 0.2 g of anhydrous sodium carbonate. The fused mass was leached with dilute hydrochloric acid (1 + 9) and transferred to the main bulk of the filtrate collected in the 250-ml flask. The solution was diluted to volume with water.

NOTE—For ores rich in iron, dissolution of sample directly in concentrated hydrochloric acid is to be preferred.

Extraction procedure

A suitable volume of sample containing about 30 μg of aluminium was pipetted into a separating funnel and 5 ml of 1% *m*/*V* tartaric acid were added, followed by 10 ml of 2% 2-isopropylquinolin-8-ol in 0.4 M hydrochloric acid. The pH was adjusted to 10 with 1 M sodium hydroxide solution, the mixture was shaken vigorously for 10 min and the precipitate was extracted into 10 ml of chloroform. After discarding the chloroform, precipitation and

extraction were repeated twice with half the amount of reagent. The aqueous phase was washed with 5 ml of chloroform and the washings were rejected. The pH of the aqueous solution was adjusted to 5 with glacial acetic acid and the aluminium was determined by quinolin-8-ol extraction as described for permanent magnet alloy.

Alumina in Manganese Ore

Manganese(II) has been found to be extracted with 2-isopropylquinolin-8-ol at pH 9.8–10.7. At this pH iron, which is normally the other matrix element present in appreciable amounts in manganese ore, is also extracted. This indicates the possibility of separation and determination of alumina in manganese ore.

The manganese ore (BCS 176/1) selected for this investigation had the following composition: manganese 49.00, iron 5.2, silica 5.6, alumina 4.1 and phosphorus 0.14%.

Preparation of sample solution

A 0.1-g amount of dried sample (dried at 110 °C) was dissolved in a mixture of 2 ml of water and 10 ml of hydrochloric acid (sp. gr. 1.18). Subsequent treatments in the preparation of the sample solution for analysis were carried out as described under Method 2 for iron ore (see above).

Procedure

An aliquot representing about 2 mg of sample was pipetted into a separating funnel and treated with 5 ml of tartaric acid (1% *m*/*V*), then 10 ml of 2-isopropylquinolin-8-ol (2% *m*/*V* in 0.4 M hydrochloric acid) were added. The pH was adjusted to 10 with 1 M sodium hydroxide solution and the mixture was gently agitated to assist precipitation and allowed to stand for 10 min. The mixture was extracted with 15 ml of chloroform, the organic phase was discarded, the aqueous solution was washed with 5 ml of chloroform and the washings were rejected. Another precipitation and extraction step was carried out as before but using only 5 ml of the reagent for precipitation and 10 ml of chloroform for extraction. After rejecting the chloroform phase the aqueous phase was washed with 10 ml of chloroform and the washings were discarded. From the aqueous solution aluminium was extracted and determined as described for permanent magnet alloy.

Aluminium in Nimonic 90 Alloy

The nimonic 90 alloy (BCS 310) had the following composition: nickel 58.75, chromium 19.22, cobalt 15.6, titanium 2.46, aluminium 1.43, iron 1.35, manganese 0.04, carbon 0.098 and silicon 0.84%.

Preparation of sample solution

About 0.1 g of the alloy was dissolved in 10 ml of concentrated hydrochloric acid in a PTFE beaker. The dissolution was completed by adding 1 ml of nitric acid (sp. gr. 1.48). The solution was diluted to 25 ml with water and gently boiled. After cooling it was transferred into a 100-ml polypropylene flask and made up to the mark with water.

Procedure

A 0.1-g amount of 2-isopropylquinolin-8-ol was weighed into a 50-ml PTFE beaker and 2 ml of the alloy solution (about 2 mg of sample) were added from a pipette. The acid in the alloy solution was sufficient to dissolve the reagent. The solution was treated with 1 ml of 35% *V*/*V* acetic acid and diluted to 15 ml with water, then heated to 60–70 °C and the pH was adjusted to 5 with 1 M sodium hydroxide solution. The precipitate was digested for 10 min, then the precipitate and the solution were transferred into a separating funnel. Quantitative transfer of the complexes was accomplished by washing with two 5-ml portions of chloroform followed by the minimum possible amount of water. The complexes were extracted into chloroform and the organic extract was discarded. Occasionally a small amount of "veil" may be formed at the interface under the conditions employed, in which event it would be necessary to retain the "veil" with the aqueous phase. The aqueous phase was transferred into the original beaker in which the first precipitation was made.

The separating funnel was washed with 5 ml of tartaric acid solution (1% *m/V*) followed by a small amount of water and the washings were collected in a beaker containing the aqueous extract. The solution was evaporated to 20 ml and treated with 5 ml of a 4% *m/V* solution of 2-isopropylquinolin-8-ol in 0.4 M hydrochloric acid.

The pH was adjusted to 10 with 1 M sodium hydroxide solution and the precipitate was digested for 10 min at 70° C. The mixture was transferred quantitatively and extracted into 15 ml of chloroform. After discarding the organic phase a third precipitation and extraction step was carried at room temperature using only half the amount of reagent used in the second precipitation and extraction. The aqueous phase was adjusted to pH 5 and washed with 5 ml of chloroform. After discarding the chloroform layer, the pH of the aqueous solution was adjusted to 11 with 1 M sodium hydroxide solution and aluminium was extracted and determined as described for permanent magnet alloy.

The aluminium extraction for the preparation of the calibration graph was carried out at pH 11.

Results and Discussion

The results obtained by the proposed method for aluminium in various British Chemical Standards samples are shown in Table II.

TABLE II

DETERMINATION OF ALUMINIUM IN BRITISH CHEMICAL STANDARDS

	Aluminium found			
Sample	Mean concentration, %	No. of determinations	Standard deviation, %	Certificate value for aluminium, %
Permanent magnet alloy, BCS 233 ..	6.98	16	0.07	6.98 (±0.08)*
Iron ore,† BCS 301	4.14	8	0.06	4.26 (±0.12)*
Manganese ore,† BCS 176/1	4.02	22	0.09	4.07 (±0.15)*
Nimonic 90 alloy, BCS 310	1.43	15	0.08	1.43 (±0.05)*

* Values in parentheses are standard deviations estimated from available information.
† Aluminium reported as alumina.

In general the agreement is good. It should be pointed out that the certified values for aluminium or alumina content represent the average results obtained independently by various procedures by different analysts. As there is no indication of the precision of the method used by different analysts, it is difficult to compare the precision of the proposed method with those of the other methods. In this situation, by using the standard deviation of the reported values for each of the test samples, an estimate of the precision for the umpire methods was obtained. By statistical tests of significance it has been shown elsewhere[6] that the estimated precision is comparable to the precision obtained by the proposed method.

The proposed method does not suffer from the complicated, time-consuming separation procedures required by methods such as mercury cathode electrolysis. Some of the methods of solvent extractions described in the literature[9–12] for the separation and determination of aluminium in samples similar in nature to those considered in this work involve the use of various masking agents and preliminary extraction agents. Preliminary extraction agents such as cupferron have been reported[3] to be unsatisfactory. In the proposed method the extraction reagents used are limited to two. The time required to complete a triplicate determination is about 3 h. One of the distinct advantages of the method is that the extraction procedure for samples such as permanent magnet alloy, manganese ore and iron ore are similar, and this allows the handling of three different kinds of samples. In this situation, with a single calibration graph three different samples can be analysed. In all of the experiments fresh calibration graphs were prepared for each batch of replicates. However, investigations showed that the slope did not alter significantly for at least 2 d if the standards were stored in the dark. In a laboratory analysing regularly these kinds of samples, this would be an additional advantage in saving time spent on the preparation of a fresh calibration graph for each batch of sample.

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Spectrophotometric Determination of Aluminium in Alloys and Ores

Part 2.* Stripping Aluminium after Chelation of Other Metals with 2-Isopropylquinolin-8-ol in Butan-1-ol

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In Part 1, aluminium was separated after extracting the other metals present, as their 2-isopropylquinolin-8-ol chelates, from aqueous solutions. In this paper a simpler and more efficient method of separation of aluminium is described. Instead of precipitating the interfering metals as their 2-isopropylquinolin-8-ol chelates and then extracting them into chloroform, they are prepared directly in butan-1-ol. Chelation of aluminium with the 2-isopropyl compound does not occur in butan-1-ol medium. The butanolic solution containing unchelated aluminium and the chelates of other metals is mixed with chloroform and then extracted with alkaline tartrate-buffered solution. In this way the uncomplexed aluminium is transferred to the aqueous phase, from which it is extracted as its quinolin-8-ol complex and determined spectrophotometrically. The technique has been applied to the determination of aluminium in complex matrices such as permanent magnet and nimonic 90 alloys.

Keywords: Chelation of metals; 2-isopropylquinolin-8-ol; butan-1-ol; solvent extraction

Development of the Method

It was pointed out in Part 1[1] that prolonged equilibration of the phases was necessary to achieve extraction when the aqueous solution of metal ions was shaken with a solution of 2-isopropylquinolin-8-ol in an organic solvent. The extraction rate is controlled by the rate of transfer of the reagent from the organic to the aqueous phase. From kinetic considerations it has been shown[2] how the low aqueous solubility of the 2-isopropyl compound can affect the rate of extraction of metal ions. Although this problem was overcome by precipitation of metal chelates from a homogeneous aqueous medium prior to their extraction, it was found that complete extraction of metal ions could be achieved only by repeated precipitations and extractions, as precipitation of the reagent decreased the concentration of the reagent available.

The limitation thus imposed by the aqueous solubility of the reagent suggested that it was desirable to consider solvents other than water in which both the metal ion and the reagent could be kept at a suitable concentration to achieve rapid and efficient chelation.

Continuous variation experiments performed to study the composition of metal chelates of 2-isopropyl- and 2-*tert*-butylquinolin-8-ols in butan-1-ol have shown that fully chelated species are present in solutions containing a sufficient concentration of chelating reagent. This indicated the possibility of preparing the metal chelates of these reagents in butan-1-ol. The advantages of using this alcohol in preference to others have been discussed elsewhere.[2] As aluminium is not chelated under the conditions used, it can be stripped from the butan-1-ol by extraction with an aqueous buffer solution.

Experimental Conditions

The chelating reagent can be added as a solid to the butanolic solution of metal salts or as a solution in butan-1-ol. If a butanolic solution is to be used, the metal residue can be dissolved directly so that the solvent could be used economically. As chelation of the 2-isopropyl homologue with some metals, such as nickel, cobalt and manganese, requires a basic medium it is necessary to use a suitable base. In practical situations the butanolic

* For Part 1 of this series, see p. 1137.

solution of metal ions would be strongly acidic so that the base will neutralise the acid and bring the pH to a value suitable for chelation to occur. Aromatic amines such as aniline or aliphatic amines such as triethylamine are soluble in butan-1-ol and can be used advantageously. Of these two bases triethylamine was chosen as it is available in a sufficiently pure state. The amount of amine that would be necessary to keep the butan-1-ol sufficiently basic was determined from blank runs conducted under the same experimental conditions as for the sample.

Stripping of Aluminium from Butan-1-ol Containing Chelates of Other Metals

The fact that butan-1-ol is only slightly soluble in water is advantageous in stripping aluminium with an aqueous buffer, but its viscous nature and low specific gravity (0.8) lead to unsatisfactory phase separation under experimental conditions. Also, as further work is to be carried out with the aqueous phase the low specific gravity of butan-1-ol creates practical difficulties. In view of these factors, it would be desirable to mix butan-1-ol containing the metal chelates with a suitable volume of chloroform, which would give a solvent of low viscosity and a higher specific gravity than water.

The aqueous buffer used to strip aluminium and the unchelated metal ions, if any, should be maintained at an optimum pH, which would prevent the extraction of chelated metal ions in favour of the aqueous phase. As 2-isopropyl chelates of many metals have been shown[2,3] to be extracted after precipitation from a basic tartrate solution between pH 9 and 12, it was decided to use a tartrate-buffered aqueous solution maintained at pH 11 to extract aluminium from the organic phase. In order to ensure that complete removal of metals interfering in the quinolin-8-ol method for aluminium was accomplished, a precipitation and extraction step was carried out on the aqueous phase according to the procedure described for the samples chosen for this investigation.

Limitation of the Method

The limitation of the method is set by the amounts of the metal ions that can be kept in solution in butan-1-ol. This limits the determination of aluminium to concentration levels of 0.5% *m/m* and above, as otherwise it would mean the use of large sample size to give a sufficient concentration of aluminium for the quinolin-8-ol method to be applicable.

Experimental and Results

Preliminary Investigations

Qualitative tests with metal perchlorates of iron, copper, nickel, cobalt, zinc and lead showed that their solution in butan-1-ol, when treated with 2-isopropylquinolin-8-ol, produced the same colour as the solution of their precipitated chelates in this solvent. With nickel and cobalt a small amount of triethylamine was added to keep the solution sufficiently basic for efficient chelation to occur. The metal chelates prepared in butan-1-ol when mixed with chloroform and extracted with water maintained at pH 10–11 were retained in the butan-1-ol - chloroform phase as indicated by the colour of the mixed solvent phase. From this it was inferred that the coloured product produced by the addition of the reagent to the butanolic solution of a metal ion was due to the formation of uncharged metal chelates of 2-isopropylquinolin-8-ol. Spectroscopic studies[2] also confirm this view.

Efficiency of Chelation in Butan-1-ol Medium

Solutions of iron, cobalt, nickel, copper, chromium and zinc ions, each containing 250 μg of metal in perchloric or hydrochloric acid (see Note 1), were evaporated nearly to dryness. To the residue were added three drops of water and the solution was treated with 5 ml of butan-1-ol containing 50 mg of 2-isopropylquinolin-8-ol. A reference solution containing no metal ions but only the appropriate acid at a concentration matching that of that present in the metal solution was also treated in the same way as the metal solutions. After the addition of the butanolic reagent, the solutions were heated (60 °C) to homogenise them. They were then treated with triethylamine. The amount of amine to be added was determined from the reference solution (see Note 2). In this solution, when all of the acid present had been neutralised the butan-1-ol turned colourless. To the solution containing metal ions two drops of amine in excess (to ensure sufficient basicity) of the amount needed for the reference solution were added. When chromium is present, the solution should be heated

on a steam-bath for 2 min after addition of the amine. The solutions were mixed with 15 ml of chloroform and extracted with 25 ml of water adjusted to between pH 10.5 and 11 and containing 5 ml of 5% tartaric acid for every 100 ml.

The pH of the aqueous phase was maintained at 10.5–11 and the concentration of alkali that must be added to tartrate-buffered aqueous solution to attain this pH range was determined from the extraction of the reference solution. The butan-1-ol - chloroform phase was discarded. The aqueous phase was washed with 10 ml of chloroform and the washings were rejected. The aqueous phase was then tested for the metal ion concerned by quinolin-8-ol extraction under suitable conditions. The chloroform extracts of quinolin-8-ol were virtually colourless and the absorbances measured at appropriate wavelengths were not significantly different from that of the blank. This indicated that chelation of these metal ions occurred quantitatively in butan-1-ol and that the chelates were retained efficiently in butan-1-ol - chloroform even after extraction with the aqueous buffer.

NOTES—

1. When a solution of chromium(III) in perchloric acid is fumed the chromium is oxidised to chromium(VI). The chromium(VI) in the presence of trace amounts of perchloric acid in the evaporated residue oxidises butan-1-ol under the conditions employed. The oxidation products of butan-1-ol are reported[2] to interfere in various ways in the extraction of chelates, particularly in presence of nickel, cobalt and other metals. For this reason, a solution of chromium(III) prepared in hydrochloric acid medium should be used.
2. In acidic butan-1-ol the reagent produces a yellow coloration. Addition of amine neutralises the acid and renders the solution colourless. Thus the reagent served as an indicator in controlling the amount of amine added.

Efficiency of Aluminium Recovery

Tests with pure aluminium solutions showed that under any conditions it remained unchelated by the 2-isopropyl compound in butan-1-ol medium also. The unchelated aluminium was quantitatively extracted from the butan-1-ol - chloroform phase into the aqueous phase. This was ascertained by carrying out separations using only aluminium standards ranging from 10 to 50 μg under conditions similar to those described for other metals. The aluminium extracted into the aqueous phase was extracted as its quinolin-8-ol chelate into chloroform. The extracts were made up to 25 ml, dried with anhydrous sodium sulphate and their absorbances were measured at 400 nm. Another set of standards of the same range were prepared by directly extracting aluminium from aqueous solution, using 1% m/V quinolin-8-ol in chloroform. The slopes of the best straight-line fit for the graphs of absorbance *versus* concentration are presented in Table I.

TABLE I

SLOPES OF CALIBRATION GRAPHS FOR ALUMINIUM

Method of preparation of standards	Slope in absorbance units per μg of aluminium in 25 ml
Butan-1-ol method	$(96.86 \pm 0.36) \times 10^{-4}$
Direct extraction from aqueous solution without using butan-1-ol	$(97.29 \pm 1.1) \times 10^{-4}$

The difference in the slopes is due mainly to errors imposed by instrumental limitations in measuring absorbance and not to differences in the recoveries of aluminium. Such a difference is expected in spectrophotometric work even if the graphs are plotted for standards prepared under similar conditions.

The efficiency of removal of metals other than aluminium by chelating them in butan-1-ol medium and the quantitative recovery of aluminium provided a rapid and simple method for determining aluminium in commercial alloys.

Determination of Aluminium in Alloys and Ores

Apparatus

Spectrophotometer.
Silica or glass cuvettes, path length 10 mm.
Separating funnels, 100 ml, *with PTFE keys.*

Reagents

Doubly distilled water and analytical-reagent grade acids were used throughout.

2-Isopropylquinolin-8-ol (synthesised material). 1 or 2% *m/V* (as required) in analytical-reagent grade butan-1-ol.

Acidic 2-isopropylquinolin-8-ol. 1 or 2% *m/V* in 0.2 and 0.4 M hydrochloric acid.

Triethylamine. Analytical-reagent grade.

Sodium hydroxide solution, 1 M. Prepared from Ultrar grade reagent.

Tartrate buffer. Every 25 ml of the buffer contained 1 ml of 5% *m/V* tartaric acid, the pH being adjusted to 11 with sodium hydroxide solution.

Quinolin-8-ol. The commercially available reagent was steam distilled and crystallised from hexane and a 1% *m/V* solution in analytical-reagent grade chloroform was prepared.

Chloroform. Analytical-reagent grade; freshly distilled if necessary.

pH indicator paper strips.

Aluminium in Permanent Magnet Alloy [British Chemical Standard (BCS) 233]

The sample solution was prepared as described in Part 1.[1]

Step 1

A suitable aliquot (≡500 μg of sample) was pipetted into a 50-ml beaker and evaporated nearly to dryness. An equal volume of a blank solution, prepared in the same way as the sample, was also pipetted and evaporated to the same extent as the sample. To each of them was added 0.2 ml of 0.5 M perchloric acid, which was allowed to spread uniformly, followed by 5 ml of butan-1-ol. The mixture was homogenised by warming, then 5 ml of 1% 2-isopropylquinolin-8-ol in butan-1-ol was added to each beaker. The blank solution turned yellow and the sample solution dark green. The blank solution was treated with triethylamine until the yellow colour was discharged. Two drops of amine in excess were added beyond this point to ensure sufficient basicity of the solution. The same amount of amine as that used in the blank was added to the sample solution. The butanolic solution of metal chelates was mixed with 15 ml of chloroform and transferred into a separating funnel, then 5 ml of chloroform were used to wash the beaker and the washings were also transferred into the funnel and mixed. The beaker was washed with 25 ml of tartrate buffer solution (pH 11) and the washings were transferred into the separating funnel. The organic and aqueous phases were shaken together to extract the uncomplexed aluminium into the aqueous phase. The pH of the aqueous phase was maintained at 10–10.5. The organic phase was separated and discarded.

Step 2

The aqueous phase was treated with 10 ml of chloroform and the chloroform layer was allowed to separate, then 5 ml of 1% *m/V* 2-isopropyl compound in 0.2 M hydrochloric acid were added and allowed to mix with the aqueous layer. The pH of the aqueous layer was adjusted to 10–10.5 with sodium hydroxide by gentle mixing without disturbing the chloroform layer. Then the solution was allowed to stand for 2 min and the pH was again ascertained. The solution was shaken vigorously to extract into chloroform any residual iron, cobalt, nickel and copper. The removal of these interfering metals was probably complete in the first step itself, but the second extraction served to ensure complete removal. After rejecting the chloroform layer the aqueous phase was washed with 5 ml of chloroform and the washings were discarded.

Step 3

From the aqueous solution aluminium was extracted successively with 10- and 5-ml portions of 1% *m/V* quinolin-8-ol in chloroform. Further treatment of the extracts was the same as described previously. The blank was taken through the same procedure as the sample. Standards with aluminium contents ranging from 10 to 50 μg per 25 ml were prepared under the same conditions as the sample. Aluminium was determined from a calibration graph of absorbance *versus* concentration.

Aluminium in Nimonic 90 Alloy (BCS 310)

The solution of the alloy was prepared as described in Part 1.[1] A suitable aliquot representing 2 mg of sample was used. Separation and determination of aluminium were accom-

plished by the same procedure as that for the permanent magnet alloy, except for the following slight modifications. In view of the increase in the sample mass the concentration of the 2-isopropyl compound used in steps 1 and 2 was 2% *m*/*V* in butan-1-ol and 0.4 M hydrochloric acid, respectively. Also, after the addition of amine in step 1 the solution was heated on a steam-bath for 2 min. This was necessary as the sample contained chromium, which chelates only when heated.

The aluminium results for the samples analysed by the proposed method are presented in Table II together with the results obtained by the precipitation and extraction technique described in Part 1.[1] Table II also gives the certified values for aluminium in these samples.

TABLE II

DETERMINATION OF ALUMINIUM IN BRITISH CHEMICAL STANDARDS

	Butan-1-ol method			Precipitation method				
Sample	Mean concentration, %	No. of determinations	Standard deviation, %	Mean concentration, %	No. of determinations	Standard deviation, %	Certified aluminium content, %	Standard deviation, %
Permanent magnet alloy (BCS 233)	7.00	11	0.16	6.98	16	0.07	6.98	0.08*
Nimonic 90 alloy (BCS 310)	1.43	10	0.08	1.43	15	0.08	1.43	0.05*

* Estimates based on available information.

Discussion and Conclusion

The results are in excellent agreement with certified values for aluminium in both alloys. The precisions of the results for the British Chemical Standards samples are only estimates based on available information.

For nimonic 90 alloy the butan-1-ol method produces results with a precision indistinguishable from that obtained using precipitation followed by extraction from aqueous solutions. Statistical analysis based on the estimated precision for the standard methods shows that the precision obtained by the butan-1-ol method is not significantly different from that of the standard methods.

For the permanent magnet alloy the results of the butan-1-ol method have a precision that is inferior to that obtained by the precipitation - extraction method. The precision is also inferior to that of the British Chemical Standards method. However, for routine purposes the precision in this instance can be considered adequate at the level of aluminium determined.

A distinct advantage the butanol method had over the precipitation extraction method is its simplicity, rapidity and that it requires only a third of the amount of chelating reagent used in the precipitation - extraction method. With these merits the method has sufficient scope for separating aluminium in iron and manganese ores and in zinc-based alloys at levels down to 0.5% *m*/*m* using only a few milligrams of sample.

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NOTE—Reference 1 is to Part 1 of this series.

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Solvent Extraction of the Thiocyanato Mixed-ligand Complexes of Iron(III) with Various Hydroxyamidines and Spectrophotometric Determination of Iron(III) in Various Biochemical and Biological Samples

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The reaction in benzene of 11 newly synthesised *N*-hydroxy-*NN'*-diarylbenzamidines (HOA) with iron(III) in the presence of thiocyanate has been investigated spectrophotometrically. The study revealed the formation of a 1:2:1 iron(III) - thiocyanate - HOA mixed complex in acidic media (0.2–0.8 M hydrochloric acid). On the basis of this sensitive colour reaction, a simple, rapid, selective and highly reproducible method for the extractive - spectrophotometric determination of microgram amounts of iron(III) in various biochemical and biological samples has been developed. The molar absorptivities of the systems are found to be between 1.1 and 1.35 $\times$ 10^4 l mol^{-1} cm^{-1} in the wavelength range 460–470 nm. The method is free of interference from most of the common metal ions and commonly used sequestering agents. The effects of experimental variables on the procedure are discussed.

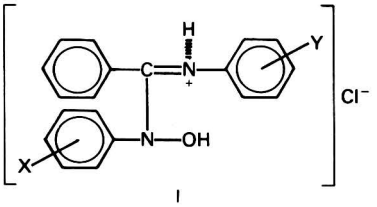
Keywords: *Solvent extraction*; *spectrophotometry*; *iron*(III) - *thiocyanate complex*; *hydroxyamidines*

Numerous methods have been reported for the spectrophotometric determination of iron in various complex materials,[1–20] and of these, the iron(III) - thiocyanate and 1,10-phenanthroline methods have been used over a long period. The iron(III) - thiocyanate method has a number of limitations, *e.g.*, variation of colour intensity with respect to the concentration of thiocyanate in addition to that of the metal, standing time, deviation from Beer's law and reaction of the thiocyanate with other elements to give coloured products. The iron(II) - 1,10-phenanthroline method is widely applicable to routine determinations of iron but it suffers from serious interference from many common ions such as nickel(II), cobalt(II), copper(II) and bismuth(III).[1] The iron(II) - 4,7-diphenyl-1,10-phenanthroline method is highly sensitive and selective but it also suffers from serious interference from copper(II), cobalt(II) and nickel(II).[5]

Other methods[2–4,6–11] that are based on the colour reaction of iron with phenols, dyes, nitroso-R salts or phenol - Rhodamine B are highly sensitive, but also suffer from serious interference from various common d-group elements. In addition, the complete extraction of the metal takes a long time and a narrow pH range is required. For these reasons, a simple, rapid and highly selective method, based on the extraction of iron(III) thiocyanate with *N*-hydroxy-*NN'*-diarylbenzamidines (HOA) in benzene, has been developed. The proposed method offers several advantages over other well known methods.[1–20] It is highly selective and can be used over a wide acidity range; it is applicable to all complex materials because most common ions do not interfere seriously; the reagent is easy to prepare and, if kept in amber-glass bottles, solutions can be stored for at least 15 d without any deterioration; and the solutions are unaffected by light, air and temperature. The additional advantages of this method over the original thiocyanate method are that the sensitivity is increased markedly and that all of the practical drawbacks have been overcome, as mentioned above.

N-Hydroxy-*NN'*-diarylbenzamidines, which are monovalent, bidentate chelating agents, have been used recently for the determination of various elements.[21–29] This work investigates the colour reaction of 11 newly synthesised *N*-hydroxy-*NN'*-diarylbenzamidines (I) with iron(III) in the presence of thiocyanate, in order to study the effect of substituents on the complexing properties of the –N = C–N(OH)– group. The spectral data for the metal chelates extracted into benzene showed that the reactions with *N*-hydroxy-*N*-(*p*-tolyl)-*N'*-(2,3-dimethyl)phenylbenzamidinium chloride is the most sensitive reagent, so this was used for the

solvent extraction and subsequent spectrophotometric determination of iron(III). The general structural formula for compounds **I** [where X = H, *m*-Cl, *p*-Cl or *p*-CH_3 and Y = H, *o*-CH_3, *m*-CH_3, *p*-CH_3, 2,3-$(CH_3)_2$, 2,5-$(CH_3)_2$, 2,6-$(CH_3)_2$ or *p*-OCH_3] is shown below.



I

Experimental

Apparatus

A Carl-Zeiss Specord ultraviolet - visible spectrophotometer and Carl-Zeiss Jena spectrocolorimeter (Spekol) with 1-cm silica cells were employed for recording the spectra and measuring the absorbance values, respectively. The pH values were determined using a Systronic pH meter, Type 322.

Chemicals and Reagents

All of the chemicals used were of analytical-reagent grade.

Standard iron(III) solution

A stock solution of iron(III) was prepared by dissolving pure iron wire (Merck) in dilute nitric acid. The oxides of nitrogen were expelled by boiling and the solution was finally diluted to 1 l with doubly distilled water.

Preparation of hydroxyamidines

Hydroxyamidines were prepared by the condensation of equimolar amounts of *N*-arylbenzimidoyl chloride and *N*-arylhydroxylamine in diethyl ether.[21] The resulting hydrochloride was recrystallised from absolute ethanol containing a few drops of concentrated hydrochloric acid. Satisfactory results were obtained for the elemental analyses.

Extraction solutions

A 5% *m*/*V* solution of potassium thiocyanate in water and a 0.2% *m*/*V* solution of the hydroxyamidine in benzene were used for all extraction work. As the solubility of the free base in benzene is higher than that of the corresponding hydrochloride, a few drops of ammonia solution were added to the solution of the hydroxyamidine in benzene, the excess of ammonia being removed by boiling.

Caution—Benzene is highly toxic and appropriate precautions should be taken.

Procedure

Place an aliquot of solution containing 50 μg of iron(III) in a 100-ml separating funnel. To this, add 2 ml of potassium thiocyanate solution and 3 ml of 5 M hydrochloric acid and then adjust the total volume of the aqueous phase to 25 ml. Equilibrate the aqueous phase with 15 ml of the solution of the hydroxyamidine in benzene for 1 min. Transfer the organic layer into a 50-ml beaker containing anhydrous sodium sulphate (2 g). Wash the aqueous phase with two 4-ml portions of fresh benzene. Transfer the combined extract into a 25-ml calibrated flask and dilute to volume with benzene. Measure the absorbance of the complex at the absorption maximum against a reagent blank as a reference.

Results and Discussion

Absorption Spectra

The absorption spectra of complexes and reagent in benzene are illustrated in Fig. 1. The red - orange complexes of iron(III) with thiocyanate and hydroxyamidines showed a sharp

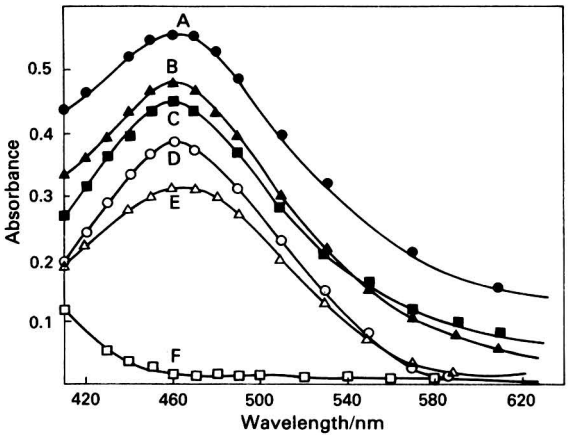


Fig. 1. Absorption spectra of the reagent and metal complexes. [SCN^-], 0.05 M; [HOA], 0.003 M; [HCl], 0.4 M. A, *N*-Hydroxy-*N*-(*p*-chlorophenyl)-*N'*-(2,3-dimethyl)phenylbenzamidine hydrochloride; [Fe], 4.29×10^{-5} M. B, *N*-Hydroxy-*N*-(*m*-chlorophenyl)-*N'*-(2,3-dimethyl)phenylbenzamidine hydrochloride; [Fe], 3.22×10^{-5} M. C, *N*-Hydroxy-*N*-phenyl-*N'*-(2,3-dimethyl)-phenylbenzamidine hydrochloride; [Fe], 3.94×10^{-5} M. D, *N*-Hydroxy-*N*-(*p*-tolyl)-*N'*-(2,3-dimethyl)phenylbenzamidine hydrochloride; [Fe], 3.58×10^{-5} M. E, *N*-Hydroxy-*N*-(*m*-chlorophenyl)-*N'*-phenylbenzamidine hydrochloride; [Fe], 2.86×10^{-5} M. F, *N*-Hydroxy-*N*-(*p*-tolyl)-*N'*-(2,3-dimethyl)phenylbenzamidine hydrochloride.

absorption maximum around 465 nm, showing that the position of the absorption maximum is not much affected by substituents. The molar absorptivity values are evidently affected by the type of substituent and the position or degree of substitution in the three phenyl rings of the hydroxyamidine.

Choice of Solvents

Various solvents, such as chloroform, carbon tetrachloride, esters, ethers, alcohols and aromatic hydrocarbons, were investigated. Of these, benzene was found to be the most suitable solvent as the extractability of the complex was very high. Toluene can also be used as a diluent but the distribution coefficient of the reagent in it is about half of that in benzene. The other solvents were found to be unsuitable for the extraction work owing to either low absorbance values or instability of the metal complexes in them.

Effect of Acidity

The acidity of the aqueous phase was maintained with 5 M hydrochloric acid (Fig. 2). Sulphuric acid was unsuitable owing to the low absorbance of the complex in it. The optimum acidity range for accurate determination of the metal was found to be 0.2–0.8 M hydrochloric acid. Therefore, in all later experimental work the acidity of the aqueous phase was adjusted to 0.6 M in hydrochloric acid in order to ensure 100% extraction of the metal.

Effect of Reagents

At least 100- and 120-fold molar excesses of hydroxyamidine and thiocyanate, respectively, are necessary for complete extraction of the metal. Addition of more hydroxyamidine (up to 0.01 M) caused no adverse effect on the position of $\lambda_{max.}$ or on the absorbance of the coloured system. However, the extraction is incomplete when the thiocyanate concentration is above

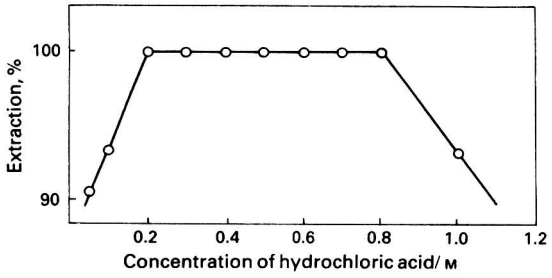


Fig. 2. Graphical representation of the effect of concentration of hydrochloric acid on extraction of Fe(III) as an Fe(III) - SCN^- - HOA complex. [Fe], 3.58×10^{-5} M; [SCN^-], 0.05 M; and [HOA], 0.003 M.

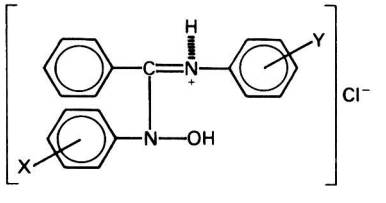
0.25 M, which may be a result of the high affinity of thiocyanate towards iron(III). The optimum concentration range of thiocyanate for 100% extraction of the metal is 0.007–0.25 M.

Effect of Substituents

In order to study the influence of substituents on the complexing properties of the ligand, 11 analogues of *N*-hydroxy-*NN'*-diarylbenzamidines were synthesised and their behaviour in the extractive separation and spectrophotometric determination of iron(III) was examined. The absorption spectra of the ternary complexes of these reagents were measured and the molar absorptivities of the coloured complexes were evaluated on the basis of iron content at their respective values of $\lambda_{max.}$ (Table I). It is observed that the presence of substituents in the *N*-phenyl or *N'*-phenyl rings has little effect on the position of $\lambda_{max.}$. However, the presence of substituents in both rings affects the molar absorptivity of the complex. The apparent trends observed are as follows: firstly, the introduction of a methyl group into the *N'*-phenyl ring had a large effect on the absorptivity of the complex and the introduction of other substituents into this ring resulted in a hyperchromic shift in the absorbance, the effect of the substituents decreasing in the order *p*-CH_3 > 2,3-$(CH_3)_2$ > *o*-CH_3 > *m*-CH_3 > 2,6-$(CH_3)_2$ ≈ 2,5-$(CH_3)_2$ > *p*-(OCH_3) (compounds 1–8); secondly, introduction of substituents

TABLE I

SPECTRAL DATA FOR IRON(III) - THIOCYANATE COMPLEXES WITH HYDROXYAMIDINES IN BENZENE



Sample No.	X	Y	$\lambda_{max.}$/nm	Molar absorptivity/ l mol^{-1} cm^{-1} $\times 10^4$	Sandell's sensitivity/ μg cm^{-2} of iron $\times 10^{-3}$
1*	*m*-Cl	H	460–470	1.10	5.07
2	*m*-Cl	*o*-CH_3	460–470	1.19	4.69
3	*m*-Cl	*m*-CH_3	460–470	1.17	4.77
4*	*m*-Cl	*p*-CH_3	460	1.23	4.54
5*	*m*-Cl	*p*-OCH_3	460	1.12	4.98
6	*m*-Cl	2,5-$(CH_3)_2$	460–470	1.16	4.81
7	*m*-Cl	2,6-$(CH_3)_2$	460–470	1.16	4.69
8	*m*-Cl	2,3-$(CH_3)_2$	460	1.20	4.65
9	H	2,3-$(CH_3)_2$	460	1.15	4.85
10	*p*-CH_3	2,3-$(CH_3)_2$	460	1.35	4.13
11	*p*-Cl	2,3-$(CH_3)_2$	460–470	1.30	4.29

* Compounds 1, 4 and 5 are free bases.

into the *N*-phenyl ring also causes a hyperchromic shift, the effect being in the order *p*-$CH_3 \approx$ *p*-Cl > *m*-Cl (compounds 8–11).

It is obvious that the introduction of methyl substituents into both of the phenyl rings attached to nitrogen atoms greatly enhanced the molar absorptivity of the complex. Consequently, the mixed complex of iron(III) - thiocyanate with *N*-hydroxy-*N*-(*p*-tolyl)-*N'*-(2,3-dimethyl)phenylbenzamidinium chloride is found to be the most sensitive.

Effect of Other Variables

Complete extraction of the metal could be achieved in 1 min and further extraction for a period of up to 30 min had no adverse effect. The extracted species was very stable and its absorbance at $\lambda_{max.}$ in benzene was constant for at least 40 h at room temperature. A variation in temperature from 20 to 40 °C and volume of aqueous phase from 15 to 60 ml had no effect on the nature of the extracted species.

Beer's Law, Sensitivity and Precision of the Method

The Sandell's sensitivities of the colour reactions lie between 0.0041 and 0.0050 μg cm^{-2} of iron with respective molar absorbances in the range 1.10–1.35 $\times$ 10^4 l mol^{-1} cm^{-1} (Table I). Beer's law is obeyed in the range 0.4–4.8 p.p.m. of iron. The optimum range for accurate determinations, as evaluated from a Ringbom plot,[30] is 0.8–3.4 p.p.m. of the metal. The precision of the method was determined for ten samples, each containing 50 μg of iron per 25 ml of solution, giving a mean value for the absorbance of 0.48 with a relative standard deviation of 0.69%.

Composition

The reaction of iron(III) with reagents in benzene is non-stoicheiometric. Therefore, a curve-fitting method[31] only was used for the determination of the ratio of metal to reagents in the complex. The slope of the graph obtained by plotting log*D* *versus* log[SCN$^-$]/log[HOA] (where *D* is the distribution coefficient of the metal), keeping other variables constant, showed the number of moles of hydroxyamidine or thiocyanate per mole of metal chelate. The iron(III) - thiocyanate - HOA ratio was found to be 1:2:1 (Fig. 3). Therefore, the composition of the neutral complex in benzene should be $Fe(SCN)_2OA$.

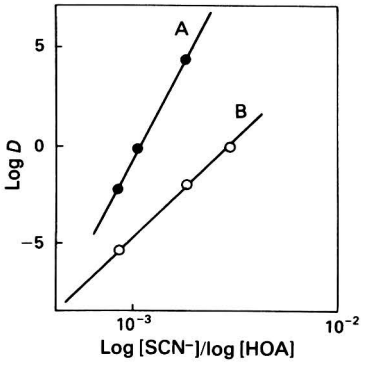


Fig. 3. Curve-fitting method for the determination of the ratio of iron(III) to HOA or SCN$^-$. [Fe], 3.58 $\times$ 10^{-5} M; [HCl], 0.4 M; [KCl], 0.1 M. A, Log *D* *versus* log[SCN$^-$]; [HOA], 0.003 M. B, Log *D* *versus* log [HOA]; [SCN$^-$], 0.05 M.

Effect of Diverse Ions

The effect of diverse ions on the determination of iron(III) was studied, as described under Procedure. At least 2500 p.p.m. of chloride, bromide, nitrate, sulphate and alkali and alkaline earth elements did not interfere in the determination of iron(III) at concentration levels of 2 p.p.m. A small amount of copper(II) is tolerated by controlling the concentration of the reagents and using thiourea as a masking agent. Silver(I) is precipitated out as a simple complex and does not interfere in the determination of the metal. The amounts of other ions tolerated are shown in Table II.

TABLE II

AMOUNTS OF DIVERSE IONS TOLERATED IN THE DETERMINATION OF IRON(III) (50 μg PER 25 ml)

Ion added	Added as	Amount tolerated,* p.p.m.	Ion added	Added as	Amount tolerated,* p.p.m.
Fe(II)	$FeSO_4(NH_4)_2SO_4.6H_2O$	800	V(V)	NH_4VO_3	50
Cu(II)	$CuSO_4.5H_2O$	50	Mo(VI)	$(NH_4)_2MoO_4$	10
Co(II)	$Co(NO_3)_2$	400	W(VI)	$Na_2WO_4.2H_2O$	40
Ni(II)	$NiCl_2.6H_2O$	500	U(VI)	$UO_2(NO_3)_2.6H_2O$	300
Mn(II)	$MnCl_2.7H_2O$	600	La(III)	$La(NO_3)_3$	1000
Zn(II)	$ZnSO_4.7H_2O$	2000	F^-	NaF	600
Cd(II)	$CdCl_2.5H_2O$	2000	I^-	KI	300
Be(II)	$BeSO_4$	1500	$S_2O_3^{2-}$	$Na_2S_2O_3.5H_2O$	100
Pb(II)	$Pb(NO_3)_2$	2000	PO_4^{3-}	Na_3PO_4	1500
Al(III)	$Al(NO_3)_3$	1000	AsO_4^{3-}	Na_2HAsO_4	1500
Cr(III)	$(NH_4)_2SO_4Cr_2(SO_4)_3.24H_2O$	800	Oxalate	$Na_2C_2O_4$	800
Bi(III)	$Bi(NO_3)_3$	1500	Citrate	$Na_3C_6H_5O_7.2H_2O$	1000
Se(IV)	Na_2SeO_3	300	Tartrate	$NaKC_4H_4O_6.4H_2O$	800
Th(IV)	$Th(NO_3)_4$	200	Acetate	$NaC_2H_3O_2$	200
Ti(IV)	$TiOC_2O_4$	100	Triethanolamine	$N(CH_2CH_2OH)_3$	400
Zr(IV)	$ZrOCl_2$	300	EDTA	$(CH_2)_2N_2(CH_2COOH)_2(CH_2COONa)_2.2H_2O$	100
			Phthalate	$KHC_8H_4O_4$	400

* Causing an error of less than 2%.

Application of Method

The proposed method was found to be applicable to the determination of iron in various biochemical and biological samples, as shown in Table III.

TABLE III

DETERMINATION OF IRON IN BIOCHEMICAL AND BIOLOGICAL SAMPLES

Sample No.	Sample	Reported iron content	Calculated iron content*: 1,10-Phenanthroline method	Calculated iron content*: Hydroxyamidine method
1	Folitrin (Biochem Pharmaceuteticals)	Iron(II) fumarate, 0.35 g	0.349 g	0.349 g
2	Foliplex (Kopran Chemicals)	Iron(II) fumarate, 0.050 g	0.050 g	0.049 g
3	Globintone (Searle)	Iron(II) fumarate, 0.200 g	0.196 g	0.195 g
4	Redieyte (Merck, Sharp & Dohme)	Iron(II) sulphate, 0.3 g	0.28 g	0.28 g
5	Calglufer (Sandoz)	Iron(II) gluconate, 0.075 g	0.071 g	0.070 g
6	Ferronicum (Sandoz)	Iron(II) gluconate, 0.325 g	0.324 g	0.324 g
7	Blood		0.057%	0.057%
8	*Spinacia oleracia* (leaves)	0.0109%†	0.0112%	0.0111%
9	*Brassica oleracia* var. *botrytis* (leaves)	0.04%†	0.041%	0.0409%
10	*Solanum tuberosum*	0.007%†	0.0065%	0.0065%

* Average of six determinations.

† Values obtained from reference 32, pp. 71, 65 and 75, respectively.

A weighed amount of the sample was transferred into a Kjeldahl flask and heated gently with a mixture of concentrated nitric and sulphuric acids (10 + 1 *V/V*) until charring commenced. Dropwise addition of concentrated nitric acid and boiling were continued until either a colourless or a pale yellow solution was obtained. This was cooled, a few millilitres of water were added and then the solution was evaporated until white fumes were evolved. The procedure was repeated two or three times. A few millilitres of dilute hydrochloric acid were added and heating was continued until fumes of nitric acid had been removed. The solution was then diluted to an appropriate volume.

An aliquot of the solution was taken and iron(III) was determined by the procedure recommended earlier. The results were compared with those obtained by the spectrophotometric method using 1,10-phenanthroline (Table III).

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Utility of π-Acceptors in Charge-transfer Complexation of Alkaloids: Chloranilic Acid as a Spectrophotometric Titrant in Non-aqueous Media

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A spectrophotometric titration method is described for the determination of some alkaloids and their dosage forms using 0.005 M chloranilic acid solution in 1,4-dioxan as the titrant. The end-point is determined by measuring the change in absorbance of the sample at 535 nm. Quantitative recoveries with good reproducibility are reported for atropine, emetine, reserpine, strychnine, yohimbine and four dosage forms. The least-squares method for the end-point location in the spectrophotometric titration is also proposed.

Keywords: *Spectrophotometric titration*; *alkaloid determination*; *chloranilic acid*; *charge-transfer complexation*

The use of chloranilic acid (2,5-dichloro-3,6-dihydroxy-*p*-benzoquinone) in the determination of metal ions has been described.[1,2] Slifkin *et al.*[3] studied the nature of complexes of chloranilic acid and amino acids and showed that a 1 : 1 complex is formed in solution and a 1 : 2 complex is formed in the solid state. On the basis of the infrared spectra of amino acids and chloranilic acid complexes, it was shown that hydrogen-bonded ionic species are formed. The spectrophotometric determination of certain alkaloids such as atropine, pilocarpine and strychnine via charge-transfer complexation with chloranilic acid has recently been reported by Elsayed and Agarwal.[4] The instantaneous formation of a stable purple 1 : 1 complex between chloranilic acid and the alkaloid prompted us to study further the use of chloranilic acid as a possible titrant, in order to devise a simple, rapid and accurate method for the determination of alkaloids as pure substances as well as in their representative pharmaceutical dosage forms. Hitherto, the use of chloranilic acid as a titrant has not been reported.

Experimental

Reagents and Materials

The following reagents were obtained from commercial sources and used as supplied: chloranilic acid (Riedel-de Haën); atropine, emetine hydrochloride and yohimbine hydrochloride (BDH); atropine sulphate, reserpine and strychnine (Merck); and strychnine hydrochloride (Sigma).

Other solvents and reagents used were of analytical-reagent grade. Dosage forms such as atropine sulphate eye drops [1% solution containing 0.002% of phenylmercury(II) nitrate, Evans Medical], atropine sulphate injections BP (0.1%, E.G.Y.T.), atropine sulphate injection BP (0.06%, Evans Medical), reserpine tablets (0.25 mg per tablet, Ciba) and emetine hydrochloride injection BP (65 mg ml^{-1}, Burroughs Wellcome) were obtained.

Chloranilic acid solution, 0.005 M. Dissolve 0.1045 g of *p*-chloranilic acid in 1,4-dioxan and make up to 1 l. The solution, when stored in an amber-glass bottle, was found to be stable for at least 6 weeks.

Alkaloid base solution. Dissolve 0.1 g of alkaloid (atropine, strychnine or reserpine) in chloroform and make up to 50 ml. Use 4–6 ml of this solution for assay as described below under general procedure.

Alkaloid salt solution. Dissolve 0.1 g of the alkaloid salt (atropine sulphate, strychnine hydrochloride, emetine hydrochloride or yohimbine hydrochloride) in about 15 ml of water in a 100-ml separating funnel. Add a few drops of dilute ammonia solution to make the solution alkaline to litmus. Extract the liberated alkaloid with 15-, 10-, 10- and 10-ml portions of chloroform. Wash each extract with the same 15 ml of water in another separating funnel. Pass each of the chloroform extracts through anhydrous sodium sulphate supported on filter-

paper in a funnel and collect the extract in a 50-ml calibrated flask and make up to the mark with chloroform. Use 4–6 ml of the extract for assay as described below under General Procedure.

Atropine sulphate eye drops solution. Transfer 10.0 ml of the solution into a 100-ml separating funnel containing 10 ml of water. Make it alkaline to litmus with dilute ammonia solution and extract the alkaloid as described above. Use 4–6 ml of the extract as described below under General Procedure.

Atropine sulphate ampoules (0.1 and 0.06%). Transfer 50.0 ml of the pooled ampoule contents into a 100-ml separating funnel and make alkaline to litmus with few drops of dilute ammonia solution. Extract the liberated alkaloid in the manner described above. Use 6–8 ml of the 0.1% atropine sulphate ampoule extract or 8–10 ml of 0.06% atropine sulphate ampoule extract for assay as described below under General Procedure.

Emetine hydrochloride ampoules (65 mg ml^{-1} solution). Transfer 2.0 ml of the solution into a 100-ml separating funnel containing 10 ml of water. Add a few drops of dilute ammonia solution to make the solution alkaline to litmus and extract the liberated alkaloid as described under *Alkaloidal salt solution*. Use 3.0–4.0 ml of the extract for assay as mentioned under General Procedure.

Reserpine tablet solution. Pulverise 30 tablets to a fine powder in a glass pestle and mortar; transfer into a dry 25-ml beaker and extract with 10 ml of chloroform. Decant through filter-paper into the titration flask. Complete the extraction of the alkaloid with three further 10-ml portions of 1,4-dioxan, decanting each portion through the same filter-paper into the titration flask. Deliver the titrant in 0.5-ml increments as described under General Procedure.

Apparatus

Absorbance measurements were made with a Bausch and Lomb Spectronic-20 spectrophotometer. The spectrophotometeric titration unit was that described by Rehm *et al.*[5] and consists of a 125-ml titration flask having inlet and outlet tubes connected by short lengths of polystyrene tubing to the inlet and outlet tubes inserted into a rubber stopper. The rubber stopper was fixed on to the absorption tube contained in the cell compartment. Homogeneity was achieved by using a magnetic stirrer. The titrant was delivered from a 10-ml burette, graduated to 0.02 ml.

General Procedure

Transfer 35 ml of 1,4-dioxan into the titration flask followed by an aliquot of the sample solution as described under Reagents and Materials. Deliver the titrant in 0.5-ml increments, stir and read the absorbance of the solution at 535 nm after each addition of titrant. The end-point is determined from a graph of absorbance *versus* volume of titrant as being the intersection of the two straight line segments, or mathematically using the equation derived by the least-squares method.

Results and Discussion

The alkaloids atropine, strychnine, reserpine, emetine and yohimbine have different structures and exhibit different absorption maxima in the ultraviolet region.[6] However, with chloranilic acid in a non-aqueous medium these alkaloids give a purple chromogen with almost similar maxima in the vicinity of 535 nm. The identical nature of the absorption spectra (Fig. 1) is probably due to the common origin of the charge-transfer complexation between the alkaloid acting as n-donor and chloranilic acid (CA) acting as a π-acceptor with the subsequent formation of a coloured anion radical of chloranilic acid (CA^-), according to the following equation:

$$R_3\ddot{N} + CA \overset{\text{Fast}}{\rightleftharpoons} \underbrace{[R_3\ddot{N} \rightarrow CA]}_{\text{Intermediate}} \rightarrow R_3\overset{+\cdot}{N} + \underbrace{CA^-}_{\text{Radical anion}}$$

1,4-Dioxan was used as the solvent owing to its low dielectric constant and it appears not to compete or shield the charge-transfer process from donor to acceptor that is necessary for instant and stable colour formation at room temperature (about 25 °C). Chloroform was used in the extraction of alkaloid dosage forms and the small proportion (usually less than 20%) that was present in the titration process did not exhibit any interference.

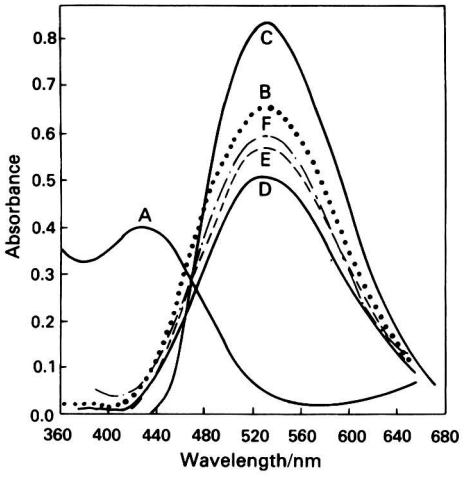

Fig. 1. Absorbance spectra of chloranilic acid, 0.35 mg ml^{-1} (A), and its complexes with: atropine, 0.15 mg ml^{-1} (B); emetine, 0.2 mg ml^{-1} (C); reserpine, 0.28 mg ml^{-1} (D); strychnine, 0.16 mg ml^{-1} (E); and yohimbine, 0.24 mg ml^{-1} (F). All in 1,4-dioxan.

The operating wavelength of 535 nm was selected as it was the wavelength of maximum absorbance for the complex and owing to the fact that there was no interference by other absorbing substances at this wavelength. The absorbance of the solution was corrected for dilution by multiplying the observed absorbance by the factor $(V_0 + v)/V_0$ where V_0 is the volume prior to the titrant addition and v is the volume of titrant added. Failure to make volume corrections may introduce unsuspected errors as extrapolation of a line of incorrect slope will give an incorrect end-point.

Fig. 2 illustrates a typical titration graph obtained with reserpine and is also representative of those obtained with atropine, strychnine and yohimbine. Initially, there is an increase in absorbance owing to complex formation but after the equivalence point is reached there is very little further increase in absorption. Fig. 3 gives the titration graph obtained with emetine. As emetine contains two basic moieties in its structure, these appear to be successively titrated as there are two breaks in the graph. The second inflection in the graph was used to determine the end-point as it gave more accurate and reproducible results compared with the calculation based on the first inflection.

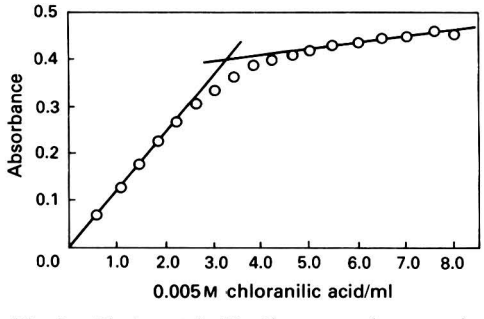

Fig. 2. Photometric titration curve for reserpine with 0.005 M chloranilic acid at 535 nm.

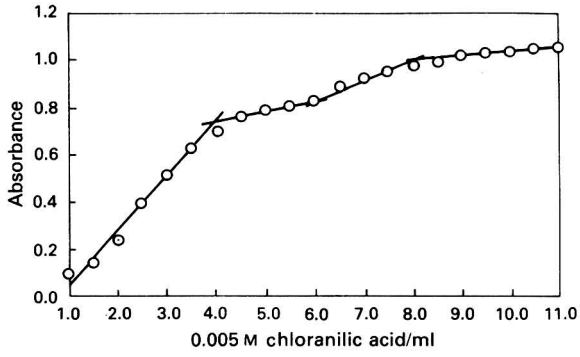

Fig. 3. Photometric titration curve for emetine with 0.005 M chloranilic acid at 535 nm.

Use of the Least-squares Method to Determine the End-point in Spectrophotometric Titrations

In spectrophotometric titration graphs, where the graphical location of the end-point may not be easy and subject to individual variation, we propose the following mathematical procedure based on the least-squares method for location of the end-point.

The absorbance readings are measured not in the vicinity of the end-point but before and after the end-point and the absorbances should give a least-squares straight line. Under such conditions at least four absorbances are measured on each side of the end-point. Using the least-squares method,[7] the regression line for the points located before the equivalence point can be described as

$$A_1 = a_1 + b_1 V_1 \quad \ldots \quad (1)$$

and after the equivalence point, the regression equation for a given line is

$$A_2 = a_2 + b_2 V_2 \quad \ldots \quad (2)$$

where A_1 and V_1 are the variable absorbances and volumes, respectively, before the end-point and A_2 and V_2 the corresponding variables after the equivalence point. The constants a_1 and b_1 are the intercept and the slope before the end-point, respectively, and a_2 and b_2 the constants after the end-point.

At the equivalence point, these two lines intersect, *i.e.*, $A_1 = A_2$ and $V_1 = V_2$. Therefore, equating the right-hand side of equations (1) and (2):

$$a_1 + b_1 V = a_2 + b_2 V \quad \ldots \quad (3)$$

Rearrangement gives

$$V = \frac{a_1 - a_2}{b_2 - b_1} \quad \ldots \quad (4)$$

where V is the volume of titrant at the equivalence point.

In equation (4)

$$a_1 = \bar{A}_1 - b_1 \bar{V}_1$$

$$a_2 = \bar{A}_2 - b_2 \bar{V}_2$$

$$b_1 = \frac{n_1 \Sigma V_1 A_1 - \Sigma V_1 \Sigma A_1}{n_1 \Sigma V_1^2 - (\Sigma V_1)^2}$$

$$b_2 = \frac{n_2 \Sigma V_2 A_2 - \Sigma V_2 \Sigma A_2}{n \Sigma V_2^2 - (\Sigma V_2)^2}$$

$\bar{A}_1$, $\bar{A}_2$, $\overline{V}_1$ and $\overline{V}_2$ are the corresponding mean values and n_1 and n_2 denote the number of absorbance measurements before and after the end-point.

Graphical and mathematical location of the end-point was applied to the data obtained in the spectrophotometric titration of atropine, strychnine and reserpine bases (Table I).

The percentage recovery was calculated using the following equation:

$$\text{Recovery (\%)} = \frac{V \times \text{molarity of chloranilic acid} \times F \times 100}{\text{Mass of sample (g)}}$$

The factor F for emetine is equal to half of the relative molecular mass as the second inflection was used for the calculation and as 1 mol of emetine reacted with 2 mol of chloranilic acid. For the other alkaloids F equals the relative molecular mass of atropine, reserpine, strychnine or yohimbine.

Statistical comparison for graphical and mathematical location of the end-point (Table I) reveals that both methods are of equal accuracy, as none of the values for $t_{calculated}$ exceed $t_{theoretical}$.

TABLE I

ASSAY RESULTS FOR ATROPINE, RESERPINE AND STRYCHNINE BASES USING GRAPHICAL AND LEAST-SQUARES METHODS FOR THE LOCATION OF END-POINT

The figures in parentheses are the mean percentage recovery ± the standard deviation

Alkaloid base	Amount taken/ mg	Graphical method		Least-squares method	
		Volume/ ml	Recovery, %	Volume/ ml	Recovery, %
Atropine	8.0	5.50	99.48	5.5859	101.04
	8.0	5.52	99.84	5.5924	101.52
	10.0	7.05	102.20	6.9712	100.87
	8.5	6.00	102.14	5.9065	100.55
	8.0	5.48	99.12	5.5042	99.49
			(100.56 ± 1.50)		(100.69 ± 0.76)
			0.83*		2.03*
Reserpine	16.0	5.20	98.91	5.2145	99.19
	16.0	5.32	101.20	5.2920	100.66
	15.0	4.65	100.37	4.9473	100.38
	12.0	3.96	100.44	4.1612	100.54
	14.0	3.94	99.93	4.5470	98.85
			(100.17 ± 0.84)		(99.92 ± 0.84)
			0.45*		0.21*
Strychnine	8.0	4.78	99.91	4.7045	98.33
	9.0	5.44	100.33	5.3887	100.11
	12.0	7.32	102.00	7.1054	99.01
	8.0	4.72	98.65	4.7505	99.29
	8.0	4.76	99.49	4.8698	101.78
	10.0	6.10	102.00	6.0612	101.35
			(100.40 ± 1.4)		(99.98 ± 1.36)
			0.86†		0.04†

* The value for $t_{calculated}$ for which $t_{theoretical}$ at $\alpha = 0.05$ is 2.776.
† The value for $t_{calculated}$ for which $t_{theoretical}$ at $\alpha = 0.05$ is 2.571.

Subjecting assay results of pharmaceutical preparations to Student's t-test, the percentage recoveries found in the dosage forms of atropine eyedrops, atropine injections (0.1%) and emetine injections were not significantly different from the label claims as $t_{calculated}$ does not exceed $t_{theoretical}$. However, in other preparations, atropine injection (0.06%) and reserpine tablets, the percentage found, although it is within the pharmacopoeial limits,[8] is significantly different from the label claim. In these instances, the values for $t_{calculated}$ exceed $t_{theoretical}$ at 95% confidence limits. Therefore, an acceptable assessment for the accuracy of the proposed method is to find the percentage recovery from a known amount (Table I, alkaloid bases and Table II, alkaloid salts) and not from the label claim. In all these instances, $t_{calculated}$ is within the limits of $t_{theoretical}$, which indicates the high accuracy of the method.

TABLE II

ASSAY RESULTS FOR SOME ALKALOID SALTS AND THEIR PHARMACEUTICAL PREPARATIONS

Preparation	n*	Amount taken/ mg	Mean recovery† ± standard deviation, %	$t_{calculated}$‡
Atrophine sulphate	5	8–10	100.59 ± 0.79	1.67
Atropine sulphate eye drops (10 mg ml^{-1})	5	8–9	100.06 ± 1.35	0.10
Atropine sulphate injection (1 mg ml^{-1})	4	7–8	101.69 ± 1.84	1.84
Atropine sulphate injection (0.6 mg ml^{-1})	4	5–6	108.82 ± 1.67	10.56
Emetine hydrochloride	6	6–10	100.56 ± 1.33	1.03
Emetine hydrochloride injection (65 mg ml^{-1})	5	7–10	100.73 ± 0.64	2.55
Reserpine tablets (0.25 mg per tablet)	4	8–10	96.35 ± 0.36	20.28
Strychnine hydrochloride	6	8–10	100.69 ± 0.97	1.74
Yohimbine hydrochloride	5	8.5–10.5	100.69 ± 1.42	1.09

* n = Number of determinations.
† Percentage recovery in alkaloid salts and percentage of label claim in pharmaceutical preparation.
‡ Values for $t_{theoretical}$ at $\alpha = 0.05$ are 3.182, 2.776 and 2.571 for 3, 4 and 5 degrees of freedom, respectively.

Auxiliary substances that are likely to be present as the preparation base, *e.g.*, starch, lactose, talc and magnesium stearate in tablets and preservatives, antioxidants or buffering agents in other pharmaceutical preparations, exhibited no interference during the assay procedure, as in the proposed method the free base is extracted prior to the instant complexation of the alkaloid with chloranilic acid.

The use of the least-squares method in the location of the equivalence point is better than the graphical method as the certainty with which the end-point is determined is increased. As indicated above, the graphical method is subjective, as in some instances different lines can be drawn through the points on the graph giving different end-point, whereas only one end-point is obtained by the least-squares method.

Our preliminary trials using chloranilic acid in potentiometric titrations of the investigated alkaloids were unsuccessful. However, attempts to find an appropriate solvent system and electrode combination are still continuing.

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Spectrophotometric Determination of Piperazine via Charge-transfer Complexes

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Iodine - piperazine or chloranil - piperazine charge-transfer complexes have been used for the sensitive assay of piperazine or its salts; these complexes exhibit intense absorption bands in the electronic spectrum. The molecular ratios of the reactants in the complexes have been established and the experimental conditions leading to maximum charge-transfer bands were also studied. The proposed procedures have been applied successfully to pure samples and drug formulations with good accuracy. The average recovery was 100.26–100.84% with piperazine - iodine and 99.33–100.33% with piperazine - chloranil charge-transfer complexes, with an average standard deviation for each method of 0.8–3.9%.

Keywords: Piperazine and piperazine salt determination; spectrophotometry; charge-transfer complexes; drug formulations

Amines are excellent electron donors, and charge-transfer complexes of these compounds with halogens and pseudohalogens have been reported.[1–3] Iodine acceptor charge-transfer complexes have been recommended in pharmaceutical analysis, *e.g.*, the complexes formed with alkaloids[4] and with ethambutol[5] have been utilised for their sensitive determination in dosage forms.

Similarly, chloranil, a π-acceptor, is known to form charge-transfer complexes and radical ions with a variety of electron donors, including amines.[1,3,6] Feigl *et al.*[7] reported that chloranil forms coloured condensation products with primary and secondary arylamines, amino acids, phenols and naphthalene. The utility of chloronil as a reagent for the spectrophotometric analysis of various amino acids has been studied by many workers[8–10]; these investigations revealed that the spectra produced were due to n - π charge-transfer complexes. Al-Ghabasha *et al.*[11] investigated the reaction of chloranil with a wide range of amines and described a method for their determination. Korany and Wahbi[12] used chloranil for the spectrophotometric determination of primary and secondary amines.

The secondary amine piperazine and its salts have therapeutic importance, and are incorporated in many pharmaceutical formulations. Gravimetric,[13–17] near-infrared spectrophotometric,[18] complexometric[19] and colorimetric[20–24] methods were reported for the determination of piperazine.

This paper describes the use of charge-transfer complexes for the sepectrophotometric determination of piperazine and its salts. The investigation involved the determination of the molecular ratios and the experimental conditions to give a maximum absorption band either in the ultraviolet region for the iodine - piperazine complex or in the visible region for the chloranil - piperazine complex.

Experimental

Reagents and Materials

The reagents used were of analytical-reagent grade and the solvents were of spectroscopic grade.

Standard solution of piperazine hexahydrate. Weigh accurately 50 mg of piperazine hexahydrate (100% purity calculated on a dry basis), dissolve it in anhydrous chloroform and dilute to 100 ml with chloroform in a calibrated flask.

Standard solutions of piperazine phosphate, adipate and citrate. Weigh accurately 50 mg of the salt (100% purity calculated on a dry basis), transfer into a 60-ml separating funnel, dissolve the powder in 3 ml of 10% sodium hydroxide solution and extract with five 15-ml portions of chloroform. Combine the chloroform extracts, dry with anhydrous sodium sulphate (5 min) and filter through dry filter-paper into a 100-ml calibrated flask. Rinse the

sodium sulphate and filter with chloroform, add the washings to the filtrate and dilute to 100 ml with chloroform.

Working solution. A working solution (for determination of piperazine using iodine) was prepared containing 0.1 mg ml^{-1} of piperazine and its salts.

Iodine solution, 4×10^{-4} M. Dissolve the required amount of iodine in chloroform.

Chloranil solution, 0.1%. Dissolve chloranil (purified by recrystallisation from acetone) in chloroform.

Instrument

Pye Unicam SP 1800 *spectrophotometer.*

Procedures

(*A*) *Piperazine - iodine*

Transfer a volume of the sample solution (containing 0.1–0.15 mg) from a microburette into a 10-ml calibrated flasks, add 4 ml of 4×10^{-4} M iodine solution and dilute to volume with chloroform. Measure the absorbance at 264 nm after 5 min against a reagent blank in a 1-cm cell.

(*B*) *Piperazine - chloranil*

Transfer a volume of the sample stock solution (containing 0.1–0.8 mg) into a 10-ml calibrated flask, add 2 ml of 0.1% chloranil solution and dilute to volume with propan-2-ol. Measure the absorbance at 545 nm after 1 h against a reagent blank in a 1-cm cell.

Assay of pharmaceutical preparations

Transfer an amount of the powdered tablet or granules or measure a volume of syrup equivalent to 50 mg of piperazine or its salts into a 60-ml separating funnel, dissolve in 3 ml of 10% sodium hydroxide solution and extract with chloroform as for the preparation of standard solutions. Dilute the chloroform extract further with chloroform to give a concentration of about 0.1 mg ml^{-1}

Transfer a volume containing 0.1 mg (*i.e.*, about 1.0 ml) into a 10-ml calibrated flask and proceed as under *A*, or transfer a volume containing 0.4 mg (*i.e.*, about 4.0 ml) into a 10-ml calibrated flask and proceed as under *B*.

Calculate the concentration of piperazine or its salts by reference to a calibration graph.

Results and Discussion

Determination of Piperazine - Iodine Charge-transfer Complex

The immediate change of the violet colour of iodine in chloroform to lemon yellow or yellowish purple upon reaction with piperazine suggested charge-transfer complex formation, and the ultraviolet region was scanned for the new band. Fig. 1 shows a hypsochromic shift of the iodine band from 520 to 264 nm with piperazine hexahydrate as a model. The Job method of continuous variation[25] indicated a molar ratio of donor to acceptor of 1:1 for the piperazine - iodine complex (Fig. 2).

In order to make use of this complex formation for the determination of piperazine, the concentration of iodine must be suitable for quantitative reaction, and should not be much higher than the piperazine concentration in order to avoid the formation of termolecular complexes with a consequent positive deviation from Beer's law, so 4 ml of 4×10^{-4} M iodine solution were adequate. Also, the absorbance should be measured 5 min after the addition of the reactants in order to minimise changes in the absorbance with time owing to conversion of the outer complex into the inner complex, the latter form being common for electron donor complexes with iodine.[1] Different solvents were tried, *e.g.*, chloroform, carbon tetrachloride, hexane and cyclohexane. The spectrum of the complex in hexane showed a low absorbance, and with cyclohexane Beer's law was not obeyed. With chloroform or carbon tetrachloride Beer's law was obeyed with a significant absorbance; chloroform was selected as the solvent.

The optimum temperature was 25 °C, as heating the complex solution decreased the absorbance. Beer's law (Fig. 3) was obeyed in the range 0.01–0.15 mg. Log ϵ (ϵ = molar absorptivity) for piperazine hexahydrate, adipate and phosphate was 3.87 and for piperazine citrate 4.19.

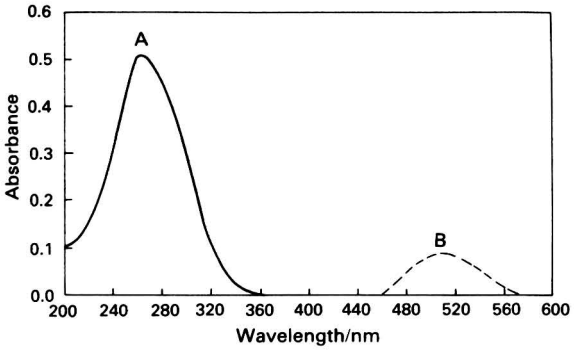

Fig. 1. Absorption spectra of A, piperazine hexahydrate (15 μg ml^{-1}) - iodine charge-transfer complex in chloroform and B, iodine - chloroform (4×10^{-4} M).

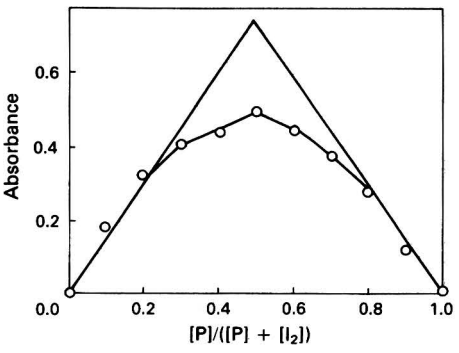

Fig. 2. Continuous variation plot of piperazine hexahydrate - iodine in chloroform (2.15×10^{-4} M).

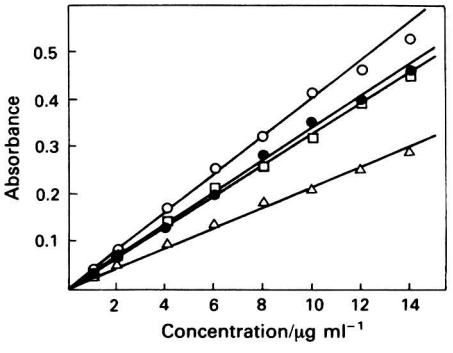

Fig. 3. Verification of Beer's law for the charge-transfer complex formed between iodine and piperazine and its salts: ○, piperazine hexahydrate; □, piperazine phosphate; ●, piperazine adipate; and △, piperazine citrate.

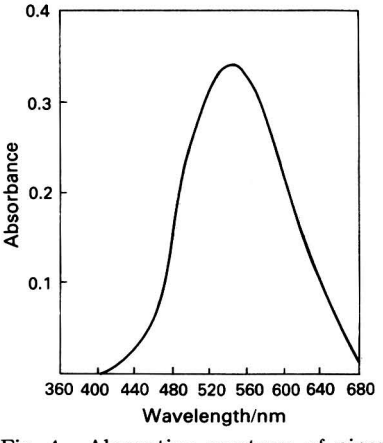

Fig. 4. Absorption spectrum of piperazine hexahydrate (50 μg ml^{-1}) - chloranil complex.

Determination of Piperazine - Chloranil Charge-transfer Complex

Chloranil in propan-2-ol - chloroform medium reacts with piperazine and its salts to form n - π charge-transfer complexes. The spectra of the complexes exhibit maximum absorption at 545 nm (Fig. 4). Because the reaction with chloranil at room temperature is slow,[10] the absorbance was measured after 1 h. Trials were made in order to accelerate the reaction by heating in a water-bath at different temperatures, but decay of the absorbance was observed. The molar ratio determined according to Job's method of continuous variarion[25] indicated a donor to acceptor ratio of 3:2 for piperazine and chloranil (Fig. 5).

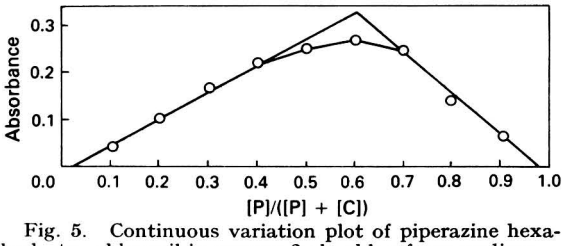

Fig. 5. Continuous variation plot of piperazine hexahydrate - chloranil in propan-2-ol - chloroform medium.

Different solvents were tried, *e.g.*, acetonitrile, ethanol, methanol and propan-2-ol - chloroform. Acetonitrile afforded a higher sensitivity than the other solvents, but it is a poor solvent for piperazine phosphate, adipate and citrate. To overcome this difficulty, the base was extracted in chloroform from alkaline medium, the chloroform evaporated under nitrogen and the residue was dissolved in acetonitrile, but unsatisfactory results were obtained. With ethanol, the reaction was examined in alkaline media using alcoholic potassium hydroxide, sodium acetate or pyridine, and the reaction failed to give a stable product. With methanol, the reaction was examined at pH 7.5 and 8.4, but a high blank reading of the same order as that of the sample was obtained. Propan-2-ol - chloroform was the solvent of choice with respect to the reaction and the stability of the complex. It was not possible to state positively that the outer complex actually takes part in the reaction but the inner complex is certainly present.[26] It is suggested that propan-2-ol - chloroform may stabilise the inner complex formed.

Beer's law (Fig. 6) was obeyed in the range 0.1–0.8 mg and 2 ml of 0.1% chloranil solution were sufficient for quantitative reaction. Log ϵ for piperazine hexahydrate, phosphate and adipate was 3.14 and for piperazine citrate 3.43. The average recovery was 99.33–100.33%.

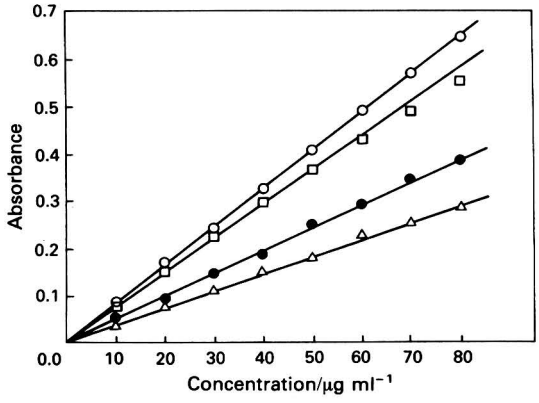

Fig. 6. Verification of Beer's law for the charge-transfer complex formed between chloranil and piperazine and its salts: ○, piperazine hexahydrate; □, piperazine phosphate; ●, piperazine adipate; and △, piperazine citrate.

TABLE I

COMPARISON OF THE RECOMMENDED PROCEDURES WITH THE OFFICIAL BP (1973) METHOD[13]

Sample	Recovery* ± standard deviation, %		
	Official method[13]	Charge-transfer complex with iodine method	Charge-transfer complex with chloranil method
Piperazine hexahydrate ..	100.00	100.3 ± 3.1	100.3 ± 0.9
Urolithine eff.-granules ..	85.6 + 0.7	83.5 ± 4.2	—†
Urosolvine eff.-granules ..	119.3 + 0.6	83.5 ± 4.2	—†
Piperazine adipate	99.2	100.3 ± 2.7	100.1 ± 1.7
Piperazine phosphate ..	99.9	99.7 ± 3.3	100.0 ± 2.1
Parazine tablet	88.43 + 0.4	99.1 ± 3.9	100.0 ± 0.00
Piperazine citrate	99.5	100.9 ± 3.2	99.3 ± 2.00
Piperazine citrate syrup ..	103.90 + 2.0	98.5 ± 1.6	109.0 ± 2.0
Vermizine syrup	112.46 + 1.8	103.3 ± 1.1	106.0 ± 0.8

* Results are the averages of at least six experiments.
† No reaction occurs.

Comparison of Methods

Table I gives the results obtained by application of both methods and an official method[13] to the determination of piperazine and its salts in pure form and in pharmaceutical preparations. The longer time and variable results are disadvantages of the official assay.[13] The results showed a standard deviation of about 0.8–3.9% for the recommended procedures, and 0.01–0.1 mg could be determined with good accuracy. Statistical parameters for the two methods are given in Table II. When a *t*-test at the 95% confidence level was applied, the calculated value of *t* did not exceed the theoretical value, which indicates that there is no significant difference between the two mean recoveries, thereby confirming the high accuracy of the two methods. The variance ratio, *F*, also reveals that there is no significant difference between the precision of the two methods.

TABLE II

STATISTICAL ANALYSIS OF IODINE AND CHLORANIL METHODS

	Piperazine hexahydrate		Piperazine phosphate		Piperazine adipate		Piperazine citrate	
Parameter	I_2 method	Chloranil method	I_2 method	Chloranil method	I_2 method	Chloranil method	I_2 method	Chloranil method
No. of experiments ..	8	6	8	6	8	6	8	6
Mean recovery, % ..	100.3	100.3	99.7	100.0	100.3	100.1	100.8	99.3
Coefficient of variation, %	9.5	0.7	11.3	4.3	7.3	3.0	10.4	4.1
Calculated value of t*	0.05		0.38		0.23		1.00	
Calculated value of F†	2.86		2.61		2.39		2.57	

* Theoretical value 1.78 ($p = 0.05$).
† Theoretical value 5.1 ($p = 0.05$).

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Simultaneous Determination of Eight Trace Elements in Human Skin by Instrumental Neutron-activation Analysis*

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Eight trace elements (scandium, chromium, iron, cobalt, zinc, selenium, rubidium and caesium) were determined simultaneously in human skin by instrumental neutron-activation analysis. The concentrations of all trace elements studied were higher in the abdominal epidermis than the dermis. Site specification was found to be essential.

Application of cluster analysis revealed that iron and zinc were the most similar trace elements in their distribution in the dermis relative to the others studied, and chromium in the epidermis was the most dissimilar.

Keywords: *Trace element determination*; *epidermis*; *dermis*; *cluster analysis*

Trace elements have been determined in human tissues by various methods and the importance of their association with many enzymes has been surveyed by Underwood[1] and Prasad.[2]

Considerable interest has been centred on trace elements that have been connected with skin diseases, *i.e.*, they may be elements used therapeutically (*e.g.*, zinc and selenium) or they may be elements that cause inflammatory reactions (*e.g.*, cobalt, nickel and chromium).

A recent survey by Iyengar *et al.*,[3] considering the available information on normal levels of trace elements in skin, disclosed some inconsistencies. This may be due to the non-specific investigations, sampling procedures and contamination or environmental and geographic variations. An example of these disparities in blood was shown in a comprehensive review by Versieck and Cornelis.[4]

The choice of analytical method depends on the elements to be determined, the matrix and the sensitivity and accuracy required. The most commonly used methods for trace element determinations in skin were (*i*) emission and absorption spectroscopy, and (*ii*) neutron-activation analysis (NAA). The detection limits attainable in method (*i*) are often a function of the level of contamination, which can be a major source of error.

Copper, manganese and zinc concentrations in human skin have been determined by Molokhia and Portnoy[5] using NAA and radiochemical separation. Molin and Wester[6] investigated the determination of the daily losses of trace elements from normal epidermis by desquamation. They determined the concentrations of eighteen elements in the epidermises of ten Scandinavian males using the Dermovac suction cup method to produce blisters on the middle part of the trunk. The determinations were made by NAA, which required lengthy radiochemical separations prior to the determination of one or more elements in the irradiated samples.

Instrumental neutron-activation analysis (INAA) has the advantage of simplicity (*i.e.*, time is not required for chemical separation) and it is ideal for routine analyses of large numbers of samples. The sample remains available for further analysis or for inter-laboratory comparison.

Cluster analysis[7] was used in this study as an aid to indicate similarities between trace elements.

The aim of this work was the simultaneous determination of eight trace elements in human dermis and epidermis. It attempted to quantify normal levels and investigate similarities in elemental distribution.

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Experimental

Samples

Skin samples were obtained from the abdomens of adult males and females of mean age 64.06 years and resident in the Manchester area. Specimens were taken within a few hours after death. Wound sites, whether surgical or accidental, were avoided. Separation of dermis and epidermis was carried out using the steam method described by Molokhia and Portnoy.[5] Great care was taken to avoid contamination of samples during either collection or storage prior to analysis. No chemicals or metal tools were used, only utensils made from Perspex or other plastics. Samples were oven-dried in Petri-dishes and stored inside "snap-on"-type polyethylene bags. In order to minimise air-borne contamination, all samples were kept inside a dust-free cabinet installed inside a clean room far from the laboratory.

Standards

Standard reference materials Nos. 1571 (orchard leaves) and 1577 (bovine liver) were prepared according to the method recommended by the National Bureau of Standards.[8] A standard kale sample was also used in the analysis.

Irradiation

Irradiation of samples and standards was carried out either at Harwell or at the North West Universities Research Reactor at Risley. The thermal neutron flux was 2×10^{12}–6×10^{12} neutrons $cm^{-2}\ s^{-1}$, and the duration of irradiation was 1 week.

Counting and Data Processing

The irradiated samples and standards were allowed to decay for 2–9 weeks as appropriate. Gamma-spectra were measured using a 90-cm^3 co-axial lithium-drifted germanium detector and a 4096-channel analyser. The data from the analyser were held on a magnetic tape. The final computer output provided digital tables of calculated peak areas using the method of Wasson.[9] These were identified and corrected for decay when necessary. Nuclear data for the trace elements are given in Table I. The experimental procedure used is shown in Fig. 1.

Statistical Analysis

The program SPSS[10] has been used for the descriptive statistics to calculate the mean, standard deviation and two-tailed significance tests. Dendrogram analyses based on the Ward's[11] method were produced using the PODS[12] program.

Results and Discussion

Fig. 2 is a schematic representation of the gamma-ray spectrum of a sample of dermis. The long decay period was sufficient to eliminate the predominant peaks of ^{24}Na and ^{42}K and to reduce the Bremsstrahlung radiation due to ^{32}P, which often prevents the measurement of low-energy peaks. Table II shows trace-element levels for pairs of epidermis and

TABLE I

NUCLEAR DATA FOR THE EIGHT TRACE ELEMENTS

Target isotope	Isotopic abundance, %	Isotope measured	Half-life	Best gamma photopeaks for determination energy/keV	Reaction
^{45}Sc	100	^{46}Sc	83.9 d	889.4	$^{45}Sc(n,\gamma)^{46}Sc$
^{50}Cr	4.32	^{51}Cr	27.8 d	320	$^{50}Cr(n,\gamma)^{51}Cr$
^{58}Fe	0.31	^{59}Fe	45.1 d	1098.6 1291.5	$^{58}Fe(n,\gamma)^{59}Fe$
^{59}Co	100	^{60}Co	5.24 years	1173.1 1332.4	$^{59}Co(n,\gamma)^{60}Co$
^{64}Zn	48.89	^{65}Zn	245 d	1115.4	$^{64}Zn(n,\gamma)^{65}Zn$
^{74}Se	0.87	^{75}Se	121 d	264.6	$^{74}Se(n,\gamma)^{75}Se$
^{85}Rb	72.15	^{86}Rb	18.66 d	1076.6	$^{85}Rb(n,\gamma)^{86}Rb$
^{133}Cs	100	^{134}Cs	2.07 years	604.7 795.8	$^{133}Cs(n,\gamma)^{134}Cs$

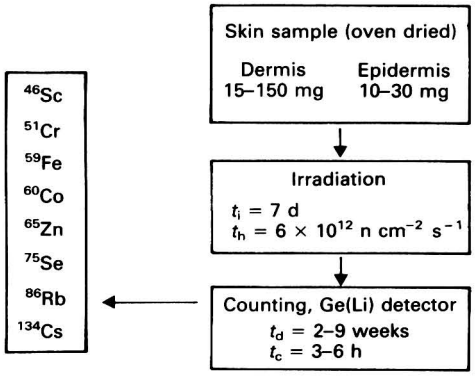

Fig. 1. Irradiation and counting scheme: t_i = irradiation time; t_d = decay time; t_c = counting time; and t_h = thermal neutron flux.

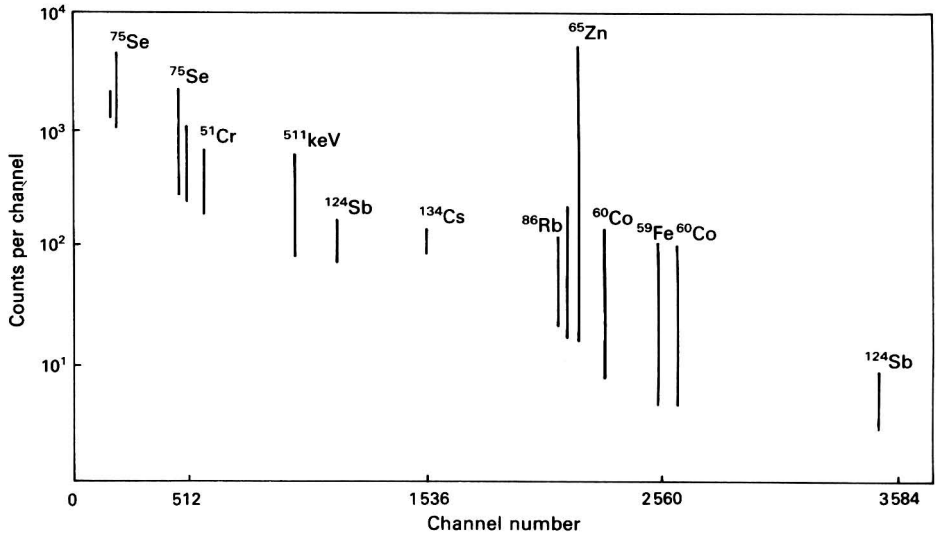

Fig. 2. Gamma spectrum of a sample of dermis. Irradiation time = 7 d. Thermal neutron flux = 6 × 10^{12} n cm^{-2} s^{-1}. Decay time = 30 d.

TABLE II

COMPARISON BETWEEN PAIRED DERMIS AND EPIDERMIS IN EIGHT TRACE ELEMENTS

Element	Number of samples	Mean concentration ± standard deviation, p.p.m. (dry mass) Dermis	Epidermis	p (2-tailed t-test)
Sc ..	6	0.0003 ± 0.0001	0.0034 ± 0.002	<0.05
Cr ..	17	0.227 ± 0.187	0.985 ± 0.948	<0.01
Fe ..	16	21.032 ± 11.128	90.171 ± 45.277	<0.001
Co ..	16	0.021 ± 0.029	0.042 ± 0.025	<0.05
Zn ..	23	13.509 ± 8.079	76.423 ± 32.171	<0.001
Se ..	25	0.345 ± 0.072	0.518 ± 0.163	<0.001
Rb ..	24	3.468 ± 1.431	6.616 ± 2.319	<0.001
Cs ..	14	0.0099 ± 0.007	0.0215 ± 0.014	<0.05

dermis of the same cutis. The difference between the mean values was tested by *t*-tests (two-tailed). For all eight trace elements studied the concentration of each element in the epidermis was higher than in the dermis and the differences were significant.

A comparative investigation was performed on another skin tissue site, samples obtained from the plantar surfaces of the feet of ten adults were analysed for iron, zinc, selenium and rubidium in both epidermis and dermis. The results are shown in Table III. Iron and rubidium showed lower concentrations in the epidermis than the dermis ($p < 0.05$). Zinc showed a lower dermal concentration ($p < 0.05$). Selenium showed a lower epidermal concentration but the difference was not significant.

TABLE III

COMPARISON BETWEEN DERMIS AND EPIDERMIS IN PLANTAR SKIN

Element	Number of samples	Mean concentration ± standard deviation, p.p.m. (dry mass)
Fe	9	38.742 ± 18.237
Fe*	9	14.387 ± 7.69
Zn	10	22.382 ± 5.362
Zn*	10	36.718 ± 14.139
Se	10	0.439 ± 0.085
Se*	10	0.343 ± 0.154
Rb	10	4.624 ± 0.843
Rb*	10	1.797 ± 0.702

* Epidermal samples. The other values refer to dermal samples.

Figs. 3 and 4 show dendrograms of trace elements in the abdominal skin of the subjects studied. In Fig. 3, at stage one, the two elements whose fusion resulted in the minimum increase in the error sum of squares formed the first group, namely zinc and iron. At the next stage selenium and rubidium fused to form a second group, and so on. Epidermal zinc then joined the group formed by iron and zinc. Finally, the two remaining groups were

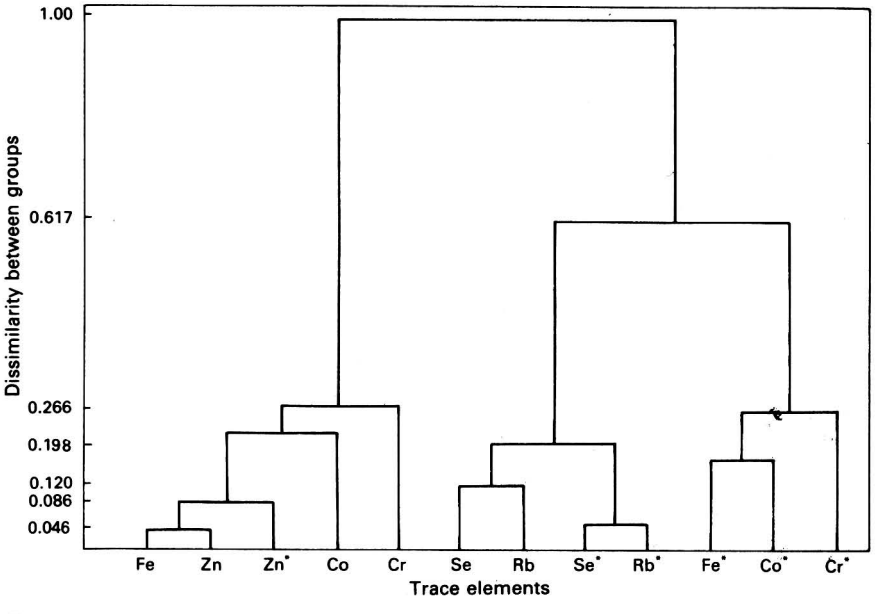

Fig. 3. Dendrogram of trace elements in dermis and epidermis. * Indicates the element in epidermis, the remaining symbols refer to the element in dermis.

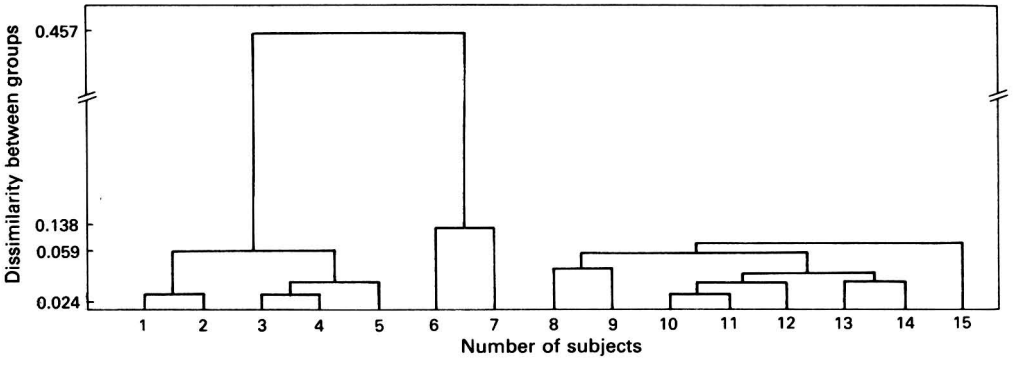

Fig. 4. Dendrogram of the subjects studied with similar distributions of trace elements.

fused to one cluster. In Fig. 4, fifteen individuals for whom all of the trace elements were measured in dermis and epidermis were examined by applying the same procedure as in Fig. 3. The group was assigned random numbers, but was arranged in increasing order for simplicity. For example, subject number 1 was found to be the most similar to subject 2, and subject number 3 was the most similar to number 4, and so on. It was hoped that a common factor would emerge to explain the similarities between these pairs of subjects. A search was carried out of the medical reports of the subjects but failed to produce any conclusive results.

Using INAA, it was possible to determine the following isotopes after selected irradiation and decay times: ^{46}Sc, ^{51}Cr, ^{59}Fe, ^{60}Co, ^{65}Zn, ^{75}Se, ^{86}Rb and ^{134}Cs. The accuracy and precision of the method were tested by repeated analyses of standard reference materials as shown in Table IV.

In Fig. 3, it was interesting to find that zinc and iron were the most similar elements; this is not surprising as both are transition elements, both are essential for life and a deficiency of either will cause ill health.

TABLE IV

ACCURACY AND REPRODUCIBILITY OF INSTRUMENTAL NEUTRON-ACTIVATION ANALYSIS

Element	Mean concentration ± standard deviation, p.p.m. (dry mass)	
	Kale	Bovine liver
Sc	0.0112 ± 0.0011*	—
	(0.0082 ± 0.001)†	
Cr	0.591 ± 0.105*	—
	(0.370 ± 0.089)†	
Fe	123 ± 8*	271 ± 27*
	(118 ± 17)†	(270 ± 20)†
Co	0.086 ± 0.012*	0.247 ± 0.031*
	(0.0624 ± 0.011)†	(0.254)†
Zn	32.04 ± 2.33*	126 ± 5*
	(33.70 ± 2.3)†	(130 ± 10)†
Se	0.123 ± 0.01*	1.03 ± 0.09*
	(0.133 ± 0.021)†	(1.10 ± 0.10)†
Rb	55.20 ± 1.8*	17.72 ± 1.8*
	(52.9 ± 4.4)†	(18.30 ± 1.0)†
Cs	0.081 ± 0.021*	0.0126*
	(0.077 ± 0.006)†	(0.019)†

* At least 10 determinations made.
† Certified[17] or best values.[18]

The use of cluster analysis may reveal unnoticed similarities between elements that might have been considered essential or non-essential. It can be seen from Fig. 3 that selenium and rubidium were similar. Selenium has recently been classified as an essential element, and its deficiency has been associated with Kesham disease.[13] Selenium toxicity has been reported by Rosenfield.[14]

Rubidium is suspected to be an essential element but very few studies have given conclusive results as pointed out by Höck[15] and Portnoy.[16]

Chromium in the epidermis was expected to be dissimilar and a contamination factor should not be excluded completely.

Further studies are planned to establish whether the abnormality of the distribution of chromium in the epidermis was due to external contamination, or to the metal not being completely adjusted by the homeostatic control mechanism.

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Method for the Simultaneous Determination of Arsenic, Aluminium, Iron, Zinc, Chromium and Copper in Plant Tissue Without the Use of Perchloric Acid

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A 0.5-g sample of plant tissue was digested with a mixture of concentrated nitric and sulphuric acids (2 + 1 *V/V*) and hydrogen peroxide. Arsenic was determined by the hydride generation method. Aluminium, iron, zinc, chromium and copper were determined by direct flame atomic-absorption spectrometry. The detection limits in dry plant material using 50 ml of aqueous solutions for analysis were 0.5 ng g^{-1} for arsenic and 0.1 $\mu g\ g^{-1}$ for aluminium, iron, zinc, chromium and copper. The relative standard deviations were 4, 6, 1, 11, 6 and 7%, respectively. All six metals were determined from the same aliquot with recoveries ranging from 93 to 118%. A study was made of the composition of the precipitate that settled out from the extracts. X-ray diffraction revealed the presence of α-aluminium oxide (corundum) and some quartz in the anti-bumping granules. α-Aluminium oxide was a source of contamination for the aluminium analysis.

Keywords: Metal determination; plant tissue; atomic-absorption spectrometry; X-ray diffraction; acid digestion

Several techniques are available for the determination of heavy metals in plants, but few are entirely suitable for the simultaneous determination of arsenic and the heavy metals at low concentrations. Dry ashing involves the combustion of organic matter in a muffle furnace and subsequent dissolution of the mineral constituents with acid. The possibility of losses of non-metals and volatile metals and failure to dissolve the ash completely must be borne in mind.[1]

Acid digestion procedures incorporate oxidising reagents to break down organic matter. The usual wet digestion procedures for plant materials use nitric - perchloric - sulphuric acid or nitric - perchloric acid mixtures. In addition to the hazardous nature of perchloric acid,[2] this acid can also produce losses of volatile elements, such as chromium, volatile as chromyl chloride[1] at 116 °C. Its use is undesirable and a procedure that obviated its use would both improve and simplify the sample preparation.

Our proposed method permits the detection of low levels of heavy metals and the simultaneous determination of arsenic, chromium, aluminium, copper, iron and zinc without using perchloric acid. The ease of the procedure makes it suitable for the routine analysis of batches of samples.

Experimental

Apparatus

A Varian, Model AA-775, atomic-absorption spectrometer equipped with a background corrector and a Philips Model PW-4025, X-ray diffraction instrument, were used.

Reagents

High-purity certified reagents were used for all analyses.

Anti-bumping granules. BDH Chemicals, lot 239563.

Nitric acid, 16 N.

Sulphuric acid, 36 N.

Hydrogen peroxide, 70% *V/V*.

Arsenic stock solution, 1000 mg l^{-1} *as* As_2O_3.

Fisher stock solution of aluminium, iron, zinc, chromium and copper, 1000 p.p.m.

Procedure

Digestion

Oven dry the plant material for 24 h at 80 °C, then pulverise it in a Wiley cutting mill to 60 mesh (0.25 mm). Weigh 0.5 g of oven-dried, milled sample into a 125-ml Erlenmeyer flask and add a few boiling chips, 10 ml of 16 M nitric acid and 5 ml of 36 M sulphuric acid. Swirl gently and digest slowly at moderate heat using a hot-plate, increasing the temperature later. Digest for 10–15 min at 90 °C. Brown fumes from the nitric acid will evolve in this period.

Increase the temperature to 170 °C and maintain it until 5 ml of solution remain, then set the flask aside to cool; the cold digest is usually colourless or occasionally yellowish. Add 2 ml of hydrogen peroxide (70% *V*/*V*) and heat gently, increasing the intensity when the initial vigorous reaction has subsided. Continue to heat until the digest has cleared, heat for a further 30 min, then leave the flask to cool. Transfer into a 50-ml calibrated flask, dilute to volume with water and mix. The concentration of residual acid is now about 10% *V*/*V*. Carry out a blank digest in the same way.

Analysis

Aliquots of the digest were analysed for aluminium, chromium, iron, copper and zinc by direct flame atomic-absorption spectrometry. Together with the samples, blanks and standards covering the range from 0.1 to 30 μg ml^{-1} of each of the above metals in 10% sulphuric acid were also run.

A set of NBS standards were digested together with the samples under the same conditions. These included orchard leaves (SRM 1571), tomato leaves (SRM 1573) and pine needles (SRM 1575).

Known amounts of solutions of zinc, copper, iron, chromium, aluminium and arsenic were added to a specified number of samples before digestion, in order to determine the recoveries with the proposed method. The blanks from acids were subtracted from the readings and were corrected for the calculations of final concentrations.

Results and Discussion

A variety of plant species were used to determine the applicability of this proposed method, including mosses (*Sphagnum capillaceum*, *S. fallax*), lichen (*Cladonia* spp.) and higher plants (*Chamaedephne calyculata*). The plant material dissolved completely in the acids and no difficulty was observed in the digestion. We observed, however, that some plant species dissolved more readily than others.

Table I presents the detection limits and statistical data for the determination of zinc, copper, iron, chromium, aluminium and arsenic in *Sphagnum fuscum*, based on ten replicate determinations. Table I also presents relative standard deviations and the mean values, and Table II shows the range of expected recoveries when samples were spiked with known amounts of standard. The analysis for arsenic was carried out using the hydride generation method.[3] Blanks and standards in 10% sulphuric acid, covering the range from 0.1 to 10 μg l^{-1}, were

TABLE I

RESULTS FOR DETERMINATION OF ELEMENTS IN *SPHAGNUM FUSCUM*

Element	Lowest detectable level/ μg g^{-1}	Adopted detectable level/ μg g^{-1}	Results for 10 replicate determinations	
			Mean/μg g^{-1}	Relative standard deviation, %
As	0.000 25	0.000 5	0.55	4
Al *	0.05	0.1	26.1	6
Fe	0.05	0.1	26.0	1
Zn	0.05	0.1	41.1	11
Cr	0.05	0.1	0.1	6
Cu	0.05	0.1	27.2	7

* Dinitrogen oxide flame.

TABLE II

RECOVERY OF METALS ADDED BEFORE DIGESTION PROCEDURE

Element	Added/ $\mu g\ ml^{-1}$	Found/ $\mu g\ ml^{-1}$	Recovery, %
Fe	1.00	1.00	100
Cu	1.00	0.93	93
Zn	1.00	0.94	94
Al	10.0	10.0	100
Cr	1.00	1.01	101
As	0.004 0	0.004 7	118

run; additions of arsenic were made before the digestion to determine the percentage recovery of arsenic, and NBS standards were analysed for arsenic contents. Table III compares the results of analyses by this method with certified values. Calibration graphs for all the analyses were in the linear range according to the manufacturers' specifications. Dilutions were necessary for arsenic as the levels were above the linear range of the method.[3]

TABLE III

RECOVERY DATA FOR NBS STANDARD REFERENCE MATERIALS

Results are means for six replicate determinations.
All results are in $\mu g\ g^{-1}$.

Sample		Concentration/$\mu g\ g^{-1}$					
		Fe	Cu	Zn	Al	Cr	As
SRM 1571 orchard leaves	Found	297	12	24	—	2.7	10.3
	Certified	300	12	25	—	2.6	10
SRM 1573 tomato leaves	Found	220	3	62.5	—	4.3	0.26
	Certified	200	3	62	—	4.5	0.27
SRM 1575 pine needles	Found	595	11	65	585	—	0.23
	Certified	600	11	—	545	—	0.21

When an aliquot of the digested plant material was allowed to stand undisturbed for several hours, a white precipitate was found to accumulate at the bottom of the flask whether the digestion was performed in perchloric - nitric - sulphuric acid or nitric - sulphuric acid mixtures. This white cloud of material started to precipitate after a short period of time. We decided to examine the precipitate to determine its constituents, and chemical structure. Aliquots of several samples of the same plant (NBS orchard leaves) were filtered through a polycarbonate membrane, pore size 3.0 μm. The precipitate was collected and dried at room temperature on the polycarbonate membrane, and further examined by X-ray diffraction.

The diffractogram obtained is shown in Fig. 1, and verifies the presence of silica (quartz)[4] (peaks 4, 6, 8 and 9) and some other minerals. Peak 3 originated from the polycarbonate membrane support, and peaks 1 and 2 showed the presence of some clay mineral; no further attempt was made to identify this clay mineral, however. Anti-bumping granules used in the extraction procedure were powdered and examined by X-ray diffraction. The diffractogram obtained is shown in Fig. 2, and represents diffraction patterns of α-aluminium oxide (corundum).[4] Peaks 1 and 2 are identical in Figs. 1 and 2, and both are part of the diffraction spectrum of the anti-bumping granules. Also, peak 5 in Fig. 1 is probably of the same origin.

Mineral quartz (silica) is known to be a constituent of some plant tissues, and may therefore originate from the plant material; however, a small amount of quartz was also present in the material used in the manufacture of the anti-bumping granules. We concluded that the α-aluminium oxide identified in the anti-bumping granules by X-ray diffraction will also cause the contamination of aluminium, as they physically separated and dissolved to some extent. No influence of other elements was indicated, good recoveries being obtained for all metals except aluminium. The digestion was repeated without using the anti-bumping granules, but using glass beads instead and a 93% recovery of aluminium was achieved.

The use of perchloric acid may cause problems, as indicated in the Introduction, and offers no extra advantage in the analysis of plants. Hydrochloric acid was not used as it would

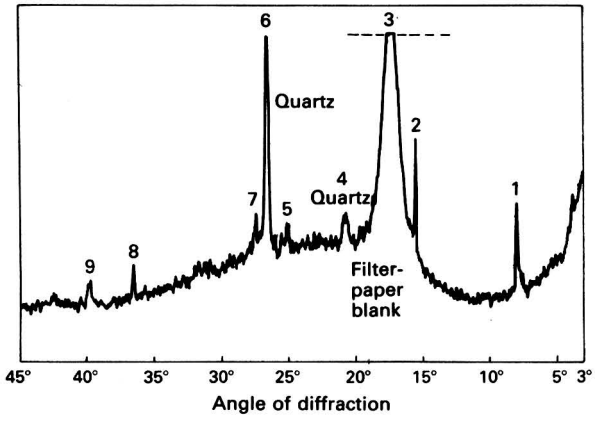

Fig. 1. X-ray diffraction pattern of NBS orchard leaves.

reduce the arsenic to form volatile arsine, which would be lost upon analysis. The combination of sulphuric and nitric acids with the application of heat will break down the cellulose structure of the plant samples, and release the metals. The hydrogen peroxide is added in order to oxidise resistant organics, and will decolorise the solution, except when there is pigmentation due to high metal concentrations. Other metals could be analysed in the same aliquot if they are naturally found at high levels; as the natural level of some trace metals is low, a concentration or extraction technique should be used.

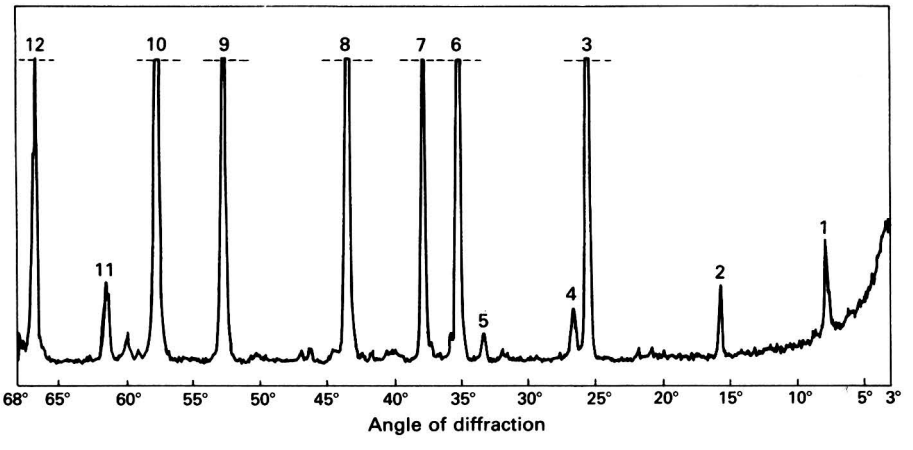

Fig. 2. X-ray diffraction pattern of BDH boiling chips, showing peaks for α-aluminium oxide (corundum) as defined by Mineral Powder Diffraction file card 10-173.[4]

Two different sample masses, low (0.5 g) and high (1.0 g), were digested. Twice the amount of nitric acid and hydrogen peroxide were used for the 1-g masses. No significant difference in the results for the two masses was found. The white precipitate in the samples could be filtered out or allowed to settle and the supernatant liquid decanted, and then analysed directly.

The proposed method provides complete dissolution of all of the elements examined in the plant structure, as demonstrated by the percentage recoveries and analyses of certified standards. The nature of the residue that contributed to the contamination with aluminium has also been demonstrated. Care must be taken in choosing the anti-bumping granules for the

acid digestion procedures. The proposed method is simple and rapid with good precision, accuracy and detection limits. Recoveries ranged from 93 to 118%, which was considered acceptable.

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Some Observations on the Capabilities of Photoacoustic Fourier Transform Infrared Spectroscopy (PAFTIR)

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The interfacing of a photoacoustic cell to a Fourier transform infrared spectrometer is described. The performance of the system with some powder and film samples has been evaluated and the differences between the photoacoustic spectra and corresponding transmission spectra are discussed. Data are presented that indicate the possibility of quantitative analysis employing the PAFTIR system.

Keywords: Photoacoustic spectroscopy; infrared spectroscopy; Fourier transform infrared spectroscopy; interferometry; polymer characterisation

Photoacoustic spectroscopy (PAS) is becoming an increasingly important technique in the examination of condensed-phase samples in the ultraviolet, visible and near-infrared spectral regions (*ca.* 250–2500 nm).[1–4] An extension of the technique into the mid-infrared region is obviously desirable, but has been hampered to date by lack of suitably intense broad-band sources. Recently, however, Rockley,[5,6] Vidrine[7] and several other workers[8,9] have shown that photoacoustic spectra can be obtained by interfacing a photoacoustic cell to interferometric spectrometer systems operating in the mid-infrared region. The increase in the signal to noise ratio (S/N) from the increased throughput of radiation, multiplexing advantage and signal averaging of interferometric systems reduce the requirement for a high intensity broad-band source.

This paper describes the interfacing of a photoacoustic cell with an interferometric system operating in the mid-infrared region of the spectrum. Differences are shown to exist between a photoacoustic spectrum for a material and its equivalent conventional absorption spectrum, and these are discussed with regard to observations made on powder and film samples.

Experimental

The FTIR spectrometer used in this work was a Nicolet 7199 (Nicolet Instrument Corp., Madison, Wisc., USA). The photoacoustic cell was a modified form of a commercially available cell (Model OAS 401, EDT Research, London). A polished potassium bromide window was substituted for the silica window normally used in the photoacoustic cell. As the photoacoustic cell detector is a capacitor microphone and its output signal is superimposed on a large d.c. voltage, a "back-off" system was used to reduce this and so avoid damage to the data processing equipment of the FTIR spectrometer. As the photoacoustic signal is weak compared with that produced by the conventional FTIR detector, an amplification stage (gain *ca.* 50) was placed between the output of the "back-off" system and the signal processing system of the FTIR spectrometer. A plane mirror was mounted in front of the beam focus in the FTIR spectrometer sampling compartment at 45° to the radiation path to direct the radiation beam vertically downwards into the photoacoustic cell. The photoacoustic cell was mounted on a foam rubber pad to isolate the cell acoustically from the interferometer optical bench, in such a position that the cell sample compartment was at the new radiation beam focus.

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Fourier transform photoacoustic infrared spectra were obtained by placing an unmodified sample in the photoacoustic cell (film samples were trimmed to fit). Unless otherwise stated, all spectra were generated from data recorded from 500 scans at 8 cm^{-1} resolution with two times zero filling and Happ-Genzel apodisation; for PAFTIR data a mirror velocity setting (VEL) of 0, equivalent to 0.055 cm s^{-1}, was used in conjunction with noise filter settings of high-pass (HPS) 0 and low-pass (LPS) **3**, with scan coincidence reliant on the intrinsic mechanical stability of the interferometer[7]; for transmission spectra, a cooled mercury cadmium telluride (MCT) detector was used, a mirror velocity setting of **34** (0.586 cm s^{-1}), noise filter settings of HPS = 1 and LPS = 5, and normal software correlation techniques were employed to ensure scan coincidence. Tables I and II give the mirror velocity settings and the frequencies corresponding to the settings of the noise filters, respectively.

TABLE I

MIRROR VELOCITY SETTINGS (VEL) ON THE NICOLET 7199 INTERFEROMETER

VEL	Velocity/cm s^{-1}
0	0.055
10	0.110
20	0.220
30	0.440
34	0.586

TABLE II

FILTER SETTINGS ON THE NICOLET 7199 FTIR SPECTROMETER

Symbol	No.	Frequency/Hz
HPS	0	10
HPS	1	100
LPS	3	2×10^3
LPS	5	10^4

Results and Discussion

The performance of the equipment was evaluated initially from the photoacoustic data presented in Figs. 1 and 2. Fig. 1 shows the average of 500 interferograms recorded from a powdered carbon black sample, and Fig. 2 represents the results obtained when two such averaged interferograms recorded sequentially were transformed and then ratioed. The amplitude of the signal from the photoacoustic detector was small in comparison with that of the conventional FTIR transmission open-beam measurement and the amplified carbon black photoacoustic signals were obtained with FTIR instrument gain settings of 1 and 128, respec-

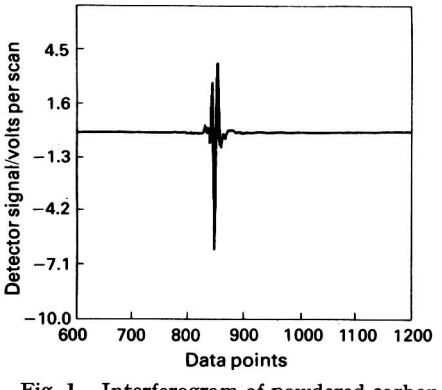

Fig. 1. Interferogram of powdered carbon black in the PA cell. VEL = 0; HPS = 0; and LPS = 3.

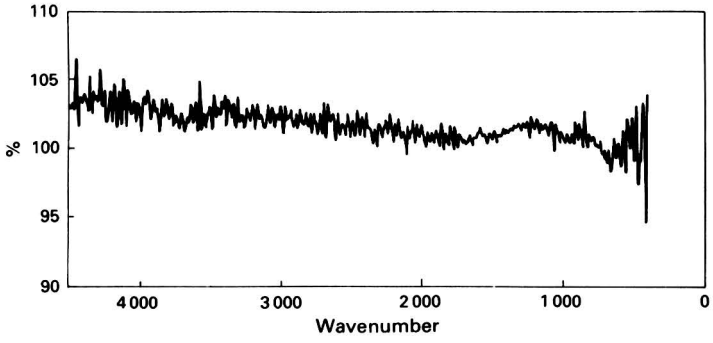

Fig. 2. Spectrum obtained from the ratio of intensities of two averaged carbon black interferograms recorded sequentially and transformed.

tively. Fig. 2 shows that there is considerable noise in the photoacoustic signal even after 500 scans. Investigation of the noise sources indicated that the most probable origin of the noise was "pick-up" on the power leads to the photoacoustic cell. This was not surprising in view of the temporary interface used. Despite these limitations, the PAFTIR spectra recorded compare favourably with those reported recently by other workers.[5–9] As a result of these preliminary measurements and taking account of the observations on performance, a photoacoustic cell purpose-built for interferometric infrared systems has been produced commercially; results obtained from this cell will be reported in a subsequent publication.

Fig. 3 shows the photoacoustic powdered carbon black single-beam energy spectra measured with the equipment described for a range of interferometer mirror velocity settings, and Fig. 4 shows the open single-beam transmission energy spectrum recorded from the FTIR system in

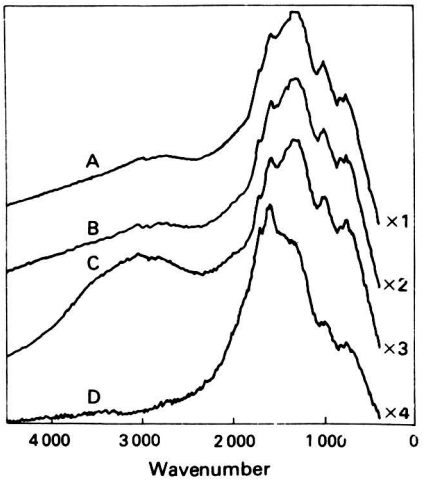

Fig. 3. PAFTIR carbon black single-beam energy spectra recorded at four different interferometer mirror velocity settings (VEL): A, 0; B, 10; C, 20; and D, 30. HPS = 0 and LPS = 3, except for D (HPS = 1 and LPS = 5).

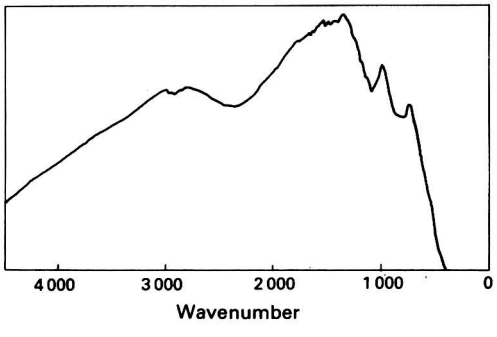

Fig. 4. Single-beam energy spectrum recorded from the FTIR system in the conventional arrangement with an MCT detector. VEL = 34; HPS = 1; and LPS = 5.

the conventional arrangement with an MCT detector. These show good agreement except for the photoacoustic data recorded at velocity setting 30, the fastest scan rate. This is noticeably attenuated at the higher wavenumbers of the spectral region and appears distorted at the peak output region. The attenuation at high wavenumbers may be caused by decreased detector response to the frequencies (2–4 kHz) at which the signals are produced in this region. The distortion at the peak output may be due to cavity resonance in the cell, which then enhances the signal at certain frequencies. It can be seen that at velocity setting 20 (0.22 cm s^{-1}) a similar increase appears in the power spectrum at a wavenumber approximately twice that of the peak output for the velocity setting 30 (0.44 cm s^{-1}) curve. Figs. 3 and 4 show that, at velocity settings in the range 0–10, photoacoustic spectra have an energy to wavenumber relationship similar to those recorded conventionally in transmission.

The similarity of PAFTIR spectra to conventional absorption FTIR spectra can be seen from a comparison of Figs. 5(*a*) and (*b*). Fig. 5(*a*) shows an absorption spectrum of a film compression moulded from a poly(vinyl chloride) (PVC) powder sample. Fig. 5(*b*) shows a PAFTIR spectrum of the powder. No shifts in band maxima positions were detected with the 8 cm^{-1} resolution used. The weak bands from *ca.* 1800 to 1500 cm^{-1} in Fig. 5(*b*) may be attributed to water vapour. They are believed to arise from water vapour in the cell possibly desorbed from the sample, as no reduction in their intensity was observed on prolonged purging of the interferometer system (for similar reasons absorptions at *ca.* 2400 cm^{-1} due to carbon dioxide may be seen in other spectra in this paper). Comparison of Figs. 5(*a*) and (*b*) show that there is in the PAFTIR spectrum a marked change in relative band intensities from those observed in absorbance (transmission), a relative increase in intensity is seen with decreasing wavenumber (increasing wavelength).

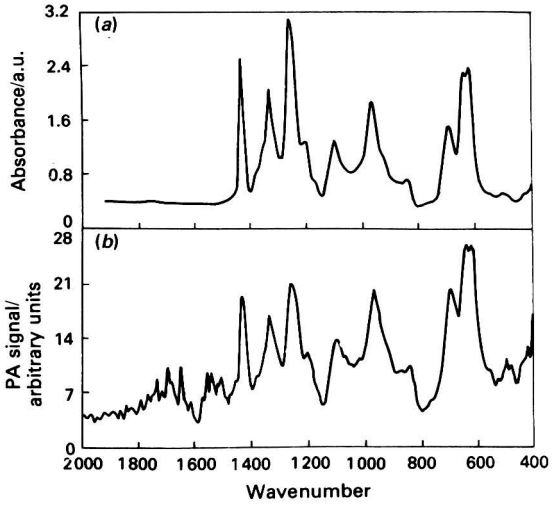

Fig. 5. (*a*) FTIR spectrum of PVC homopolymer film; and (*b*) PAFTIR spectrum of PVC powder. PA = photoacoustic.

The acoustic signal in condensed-phase PAS is produced by the diffusion of a thermal wave from the solid sample generated by absorption of a modulated radiation beam. In conventional PAS, modulation is generally at constant frequency and generated mechanically, *e.g.*, by a rotating sector that periodically interrupts the beam. In PAFTIR spectroscopy all radiation frequencies are modulated simultaneously, and each wavenumber ($\bar{\nu}$ cm^{-1}) is modulated at a frequency (ω Hz) given by

$$\omega = 2\,\bar{\nu}\,v \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where v (cm s^{-1}) is the interferometer mirror velocity.

Hence the modulation frequency at each wavenumber is different and, as can be seen from equation (1), at lower wavenumbers the modulation frequency is lower.

Rosencwaig and Gersho[10] have proposed a theory for the photoacoustic effect in solids in which they assume that the sample is a continuum, and consider the dependence of photoacoustic signal on modulation frequency for distinct cases. The significant feature of each case is the relationship between the thermal diffusion depth (μ_s) of a particular material and its optical absorption length (μ_β). In addition, for powders, several other factors need to be considered, *e.g.*, particle size to wavelength ratio, scattering/radiation penetration and the effect of interparticle gas near the surface. Indeed, Nordal and Kanstad[11] showed an approximately 2-fold increase in the 10.26-μm PAS band intensity for ammonium sulphate powder when the grain size was decreased from 1000 to less than 100 μm. However, in recent PAFTIR measurements that we have made over a narrower range of powder particle sizes (<63, 63–106, 106–150, 150–200, >200 μm) on PVC and vinyl chloride - vinyl acetate copolymers, no gross differences related to particle size were observed within the limits of our experimental signal to noise ratio. We also measured the PAFTIR spectra of films (nominal thickness 0.3 mm) compression moulded from the bulk powders, and no significant differences were observed within a spectrum in relative band intensities between corresponding film and powder PAFTIR spectra. Thus, although there is at present no satisfactory complete theory for the photoacoustic spectra of powder samples, because in these instances particle size did not appear to be a large contributory factor to band intensity, we have applied the Rosencwaig and Gersho calculated modulation dependence to the data presented in Fig. 5(*b*).

The Rosencwaig and Gersho (RG) model predicts that the magnitude of the photoacoustic signal will vary with modulation frequency, and a number of specific cases are given in this model. A polymer sample such as that considered in Fig. 5(*b*) has a very low bulk thermal conductivity; therefore, we can assume that the sample is thermally thick, *i.e.*, the thermal diffusion depth (μ_s) (the maximum depth from which heat will diffuse detectably from the sample) is less than the thickness of the sample. As infrared absorption coefficients (β cm^{-1}) are generally considerably lower than those in the ultraviolet and visible regions, we have assumed that for most wavenumbers in this spectral region the optical absorption length ($\mu_\beta = 1/\beta$) will be greater than the thermal diffusion depth. In the RG case for "thermally thick solids" where $\mu_s < \mu_\beta$, the magnitude of the photoacoustic signal is shown to be proportional to $\omega^{-3/2}$. However, the reference material carbon black can be considered photoacoustically saturated, *i.e.*, $\mu_s > \mu_\beta$, hence the signal amplitude is independent of β. In this instance the RG treatment shows the photoacoustic signal to be proportional to ω^{-1}.

In PAFTIR, spectral normalisation at each wavenumber value is achieved by the ratio of the sample signal, P_s, to the carbon black reference signal, P_c. If P_n is the normalised photoacoustic signal, then

$$P_n = \frac{P_s}{P_c} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

but $P_s \propto \omega^{-3/2}$ and $P_c \propto \omega^{-1}$, and therefore

$$P_n \propto \omega^{-1/2} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Thus, in a normalised PAFTIR spectrum, a change in signal intensity with respect to modulation frequency might be corrected for by reference to a particular wavenumber (R) through the equation

$$\frac{P_x}{P_R} = \frac{P^1{}_x}{P^1{}_R}\left(\frac{\omega_x}{\omega_R}\right)^{\frac{1}{2}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where $P^1{}_x/P^1{}_R$ is a band intensity ratio as measured from the normalised PAFTIR spectrum between wavenumber x and reference wavenumber R, ω_x is the modulation frequency at wavenumber x, ω_R is the modulation frequency at reference wavenumber R, P_x/P_R is the PAFTIR band intensity ratio corrected with respect to change of modulation frequency and ω_x and ω_R are calculated from equation (1).

To test this relationship, the normalised intensities of five bands in the PAFTIR spectrum shown in Fig. 5(*b*) were ratioed to the intensity of the band at 638 cm^{-1}. The measured ratios were then corrected for the change in modulation frequency as in equation (4), and compared

with corresponding absorption band intensity ratios (A_x/A_R) measured from the conventional FTIR spectrum shown in Fig. 5(*a*). Using the notation of equation (4), Table III shows the measured and corrected PAFTIR band intensity ratios together with those calculated from the FTIR absorbance spectrum.

TABLE III

MEASURED AND CORRECTED PAFTIR BAND INTENSITY RATIOS AND CALCULATED VALUES

Reference parameters: $R = 638$ cm^{-1}; $\omega_R = 70.2$ Hz, $P^1{}_R = P_R = 21.86$; and $A_R = 1.90$.

x/cm^{-1}	ω_x/Hz	Measured PAFTIR band intensity, $P^1{}_x$	FTIR absorbance, A_x	$\frac{P^1{}_x}{P^1{}_R}$	$\frac{P_x}{P_R}$	$\frac{A_x}{A_R}$
1431	157.4	15.3	2.15	0.70	1.05	1.13
1331	146.4	12.9	1.70	0.59	0.85	0.89
1250	137.5	17.1	2.73	0.78	1.09	1.43
1100	121.0	6.6*	0.73*	0.30	0.39	0.38
963	105.9	10.5*	1.13*	0.48	0.59	0.59

* Magnitude obtained using a sloping base-line correction; all other magnitudes were obtained with a horizontal base line.

Although we have neglected many factors that may affect photoacoustic signal intensities, the results show good agreement between the corrected intensity ratios for the PAFTIR spectrum and the corresponding absorbance ratios calculated from the conventional FTIR spectrum. The only major deviation is for the corrected ratio calculated from the most intense band at 1250 cm^{-1}, this being considerably smaller than its absorbance ratio equivalent. One reason for this deviation may be that the absorption coefficient is sufficiently great to shift the sample at this wavenumber into a different case in the RG model, where $\mu_s > \mu_\beta$, when the analysis given above would become invalid.

These results suggest that, at least for this sample, the RG prediction predominates and variation of physical form plays a relatively minor role in photoacoustic signal production in the mid-infrared region. Hence it might be reasonable to suppose that very little, if any, of the distortion in Fig. 5(*b*) compared with Fig. 5(*a*) is caused by the change in the scattering power of the sample as the wavelength of the incident radiation varies, and the observed increase in signal with decreasing wavenumber (increasing wavelength) may be predicted from the RG model.

Figs. 6(*a*) and (*b*) illustrate the use of PAFTIR to facilitate the analysis of opaque samples. Fig. 6(*a*) shows the transmission spectrum of a black polymer film detached from a "non-stick" coating on the inside of a spray-gun paint reservoir, and Fig. 6(*b*) shows the PAFTIR spectrum of the film while still retained on the aluminium backing of the utensil. In both instances the band at 980 cm^{-1} is clearly observable, indicating that the material is a tetrafluoroethylene copolymer. The transmission spectrum, however, could only be obtained by first etching the 3-mm backing of metal from the sample, whereas the PAFTIR spectrum was obtained from a sample of the paint reservoir trimmed to fit the photoacoustic cell.

It should be pointed out that for our PAFTIR spectrum of the black polymer film in Fig. 6(*b*), an increase in band intensity with decreasing wavenumber is no longer evident. Indeed, if we ignore the very strong C–F stretching bands near 1200 cm^{-1}, where saturation is almost certainly occurring, the band intensities of the 980, 630 and 515 cm^{-1} bands show a marked decrease with decreasing wavenumber compared with the absorbance spectrum in Fig. 6(*a*). The analysis may be complicated as we cannot be sure that the two films are identical, detachment from the aluminium base may have modified the material examined in transmission, optical and thermal effects of the backing may contribute to the photoacoustic signal and the effect of the carbon black filler in the film is unknown. However, this suggests that considerable care will have to be used in the quantitative interpretation of PAFTIR spectra, and a much more detailed examination of factors that may affect the production of photoacoustic signals is required.

Figs. 7(*a*)–(*c*) show the PAFTIR spectra of a PVC homopolymer, and two vinyl chloride - vinyl acetate copolymers containing 8.1 and 13.8% of vinyl acetate as copolymer. The

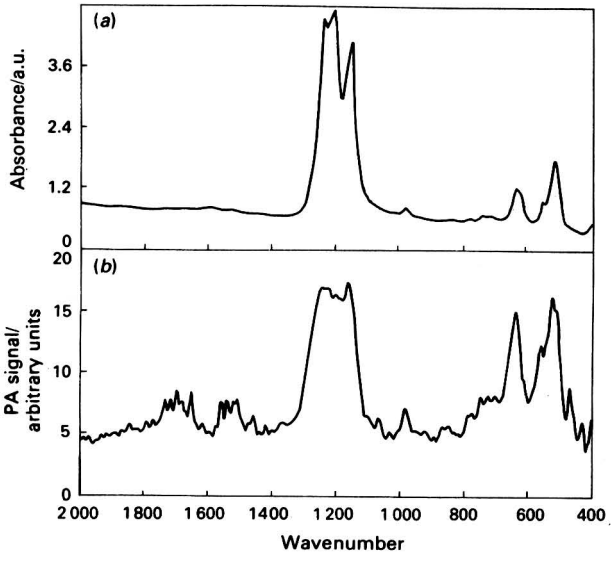

Fig. 6. (*a*) FTIR spectrum of "non-stick" coating removed from the inside of a spray gun paint reservoir; and (*b*) PAFTIR spectrum of the "non-stick" coating with the metal backing still present.

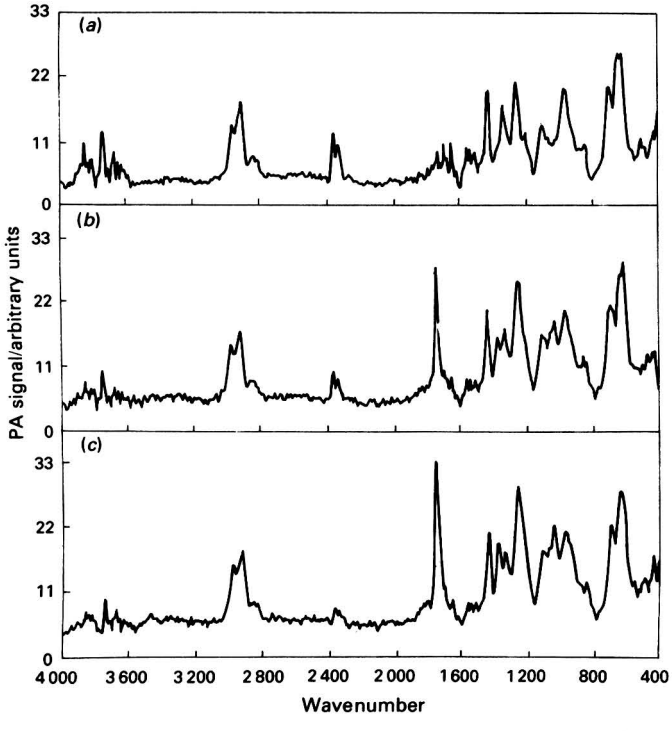

Fig. 7. (*a*) PAFTIR spectrum of PVC powder; (*b*) PAFTIR spectrum of vinyl chloride - vinyl acetate (8.1%) copolymer powder; and (*c*) PAFTIR spectrum of vinyl chloride - vinyl acetate (13.8%) copolymer powder.

increase in the carbonyl band intensity at 1740 cm^{-1} with increasing copolymerised vinyl acetate content is marked and, although the use of only three samples gives too few data to establish true quantitative information, it can be seen that, in addition to illustrating clearly the presence of vinyl acetate in the copolymers, the spectra potentially allow the classification of the samples in order of increasing copolymer content.

Conclusion

Photoacoustic spectroscopy using a photoacoustic cell in conjunction with an FTIR spectrometer has been shown to be a useful technique for the study of a number of different types of sample in the mid-infrared region. Analysis of the limited number of spectra recorded suggests that in some instances the quantitative differences observed between photoacoustic and conventional transmission spectra may be accounted for along the lines of the Rosencwaig and Gersho theory for the PAS of condensed phase samples, and thus analytical use may be made of the spectra. However, caution should be exercised in interpreting spectra of discontinuous samples and powders. If an improvement in attainable signal to noise ratios can be achieved, PAFTIR spectroscopy could prove simpler and more advantageous than currently available techniques for such samples. In addition, there is evidence that at least semi-quantitative results can be obtained. In the ultraviolet, visible and near-infrared regions, quantitative methods have already been established for solids[4,12] and this indicates that such methods might be extended to analysis in the mid-infrared region of the electromagnetic spectrum.

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Pattern Display for Characterisation of Trace Amounts of Odorants Discharged from Nine Odour Sources

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The odorants discharged from nine odour sources were classified into eight compound groups and were analysed by a systematic gas-chromatographic technique. The characterisation of trace amounts of the odorants was carried out by using the values for new proposed units (pOU_m, pOU_a, logOU, OU_t and OU; all terms are dimensionless) based on the ratio of the detected concentration to the odour recognition threshold concentration. The graphical representation of these data is effective for rapid recognition of the whole state. A polar co-ordinate pattern display was also proposed for the explanation of the relationship between odour characteristics (odour quality and intensity) and chemical analysis data of the odorants responsible for each odour discharged from nine odour sources. The calculated pOU_m and pOU_a values of eight odorant groups were plotted on polar co-ordinate circular odour charts. These charts illustrated a characteristic pattern and it was found that the shapes and sizes of each odour chart could characterise the quality and intensity of each odour from the nine odour sources. This was confirmed by investigating examples of processes or factories belonging to the nine odour sources.

Keywords: Odorant characterisation; air analysis; gas chromatography; cold- and adsorption-trapping; pre-column concentration

In recent years, several modern instrumental analytical techniques, *e.g.*, gas chromatography, mass spectrometry, gas chromatography - mass spectrometry, Fourier transfer infrared spectroscopy and nuclear magnetic resonance spectroscopy, have been developed for the separation, detection and identification of individual compounds in simple and complex mixtures. The detection limits of these techniques have been increased to approach the sensitivity of human olfaction. In several methods, it is interesting that human olfaction can be used as the detector at the exit port of the gas chromatographic column to determine the odorants. The combination of a pre-concentration technique and a detection method for the determination of the most important compounds responsible for odours or aroma and flavour is widely used in many fields, *e.g.*, foods, cosmetics, agriculture, air pollution and odour sources.[1–8]

Fuller *et al.*[9] originally developed the technique of combining a detection method with gas chromatography. Guadagni *et al.*[10] used the combination technique to assess the role of various aroma fractions in apple essence. If information on the chemical nature of odours was available it would be valuable for the control and deodorisation of odour sources. However, little is known about the chemical nature of odours at sites of air pollution or at odour sources, and there are few reports of simple chemical characterisation procedures for odours from various sources.

In this paper the simple chemical characterisation of trace amounts of odorants responsible for the odours discharged from nine odour sources (*viz.*, a sewage treatment plant, a fishmeal factory, a shell-mould processing plant, a night-soil treatment plant, a metal paint dryer, a kraft pulp recovery boiler, kraft pulp wastewater, a poultry manure dryer and a corn-starch manufacturing works) is described. The odorants were classified into the following eight groups of compounds: group (I), sulphur compounds; group (II), lower aliphatic amines; group (III), carbonyl compounds; group (IV), hydrocarbons; group (V), lower aliphatic monohydric alcohols; group (VI), phenols; group (VII), lower fatty acids; and group (VIII),

indoles. To explain the relationship between the odour characteristics and the analytical data for the particular odorants discharged from the nine odour sources, new units were proposed (pOU_m, pOU_a, logOU, OU_t and OU; all are dimensionless), where the odour units (OU) are based on the ratio of the detected concentration to the odour recognition threshold concentration. For each odour source, the values of the new proposed units, pOU_m, pOU_a, logOU, OU_t and OU, for one set of odorants were calculated from the following equations in order to characterise the eight odorant groups:

$$pOU_m = \log OU_1 + \log OU_2 + \ldots + \log OU_n \quad (1)$$

$$pOU_a = \log OU_t \quad (2)$$

$$OU_t = OU_1 + OU_2 + \ldots + OU_n \quad (3)$$

where logOU represents the logarithm of the odour unit of each detected odorant; pOU_m represents the total effect of adding all the logOU values of the detected odorants in one odorant group (*i.e.*, multiplying the OU values); pOU_a is the logarithm of the total of sum of all the OU values of the detected odorants in one odorant group; OU_t is the sum of all OU values in one odorant group; and OU_1, OU_2, . . ., OU_n represent the ratios of the detected concentrations (C_n, in p.p.b.) of the detected odorants to the corresponding odour recognition threshold values (R.Th.V.$_n$, in p.p.b.) of the respective detected odorants. All of the odour recognition threshold values used in this study have been cited previously, and the values were determined in an odour-free room.[11–14]

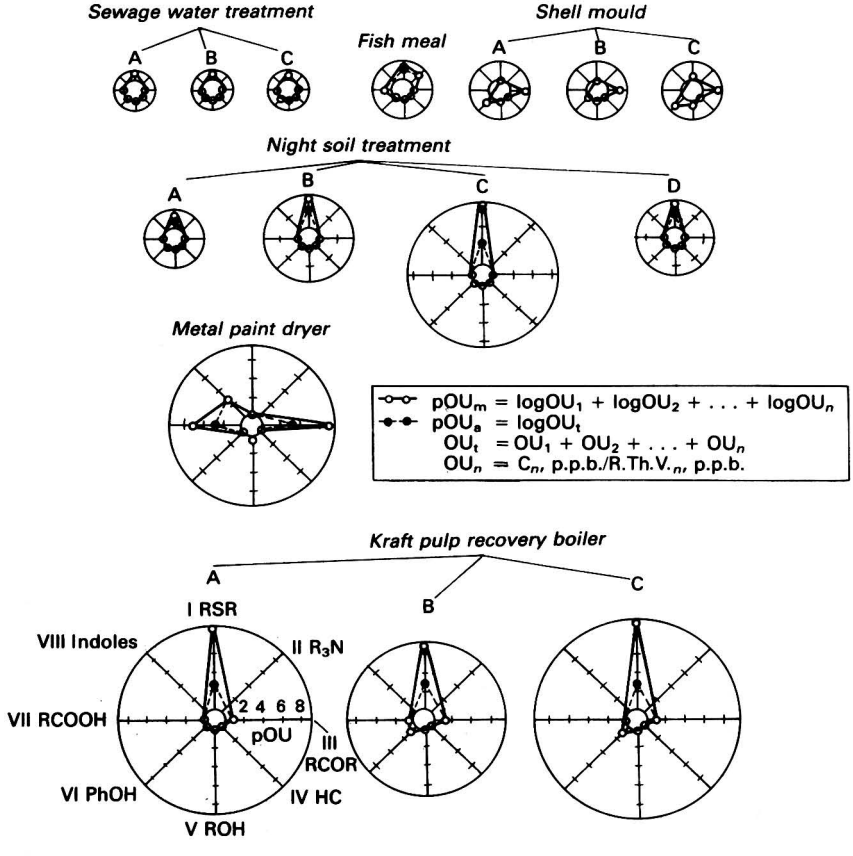

Fig. 1. Circular odour charts of pOU detected for 8 odorant groups in sample gases from some odour sources. HC represents hydrocarbons.

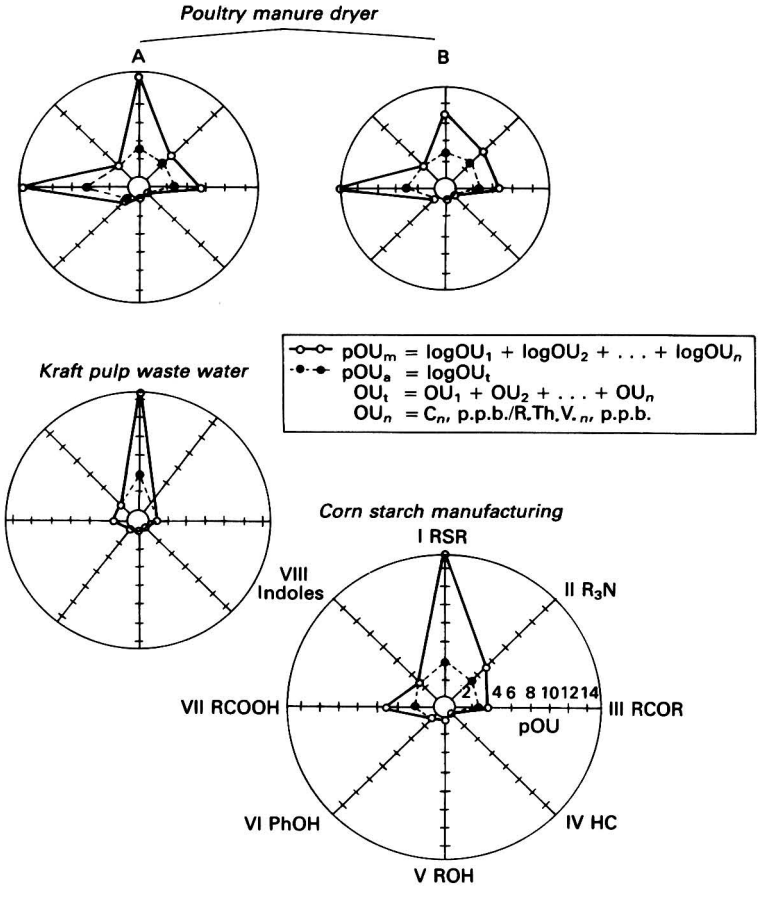

Fig. 2. Circular odour charts of pOU detected for 8 odorant groups in sample gases from odour sources. HC represents hydrocarbons.

Experimental

Gas Chromatography

Gas chromatography was used for the determination of trace amounts of odorants from the nine odour sources using two pre-concentration methods, namely, cold trapping with liquid argon for odorant groups (I) to (V) and room-temperature adsorption trapping with porous polymer beads, such as Tenax-GC or graphitised carbon black (Supelco, Bellefonte, Pa., USA) coated with a small amount of stationary phase (FFAP - orthophosphoric acid) [0.1% + 0.1% on 80–100 mesh Carbopak C (Wako Pure Chemical Industries, Osaka, Japan)], for odorant groups (VI) to (VIII). The design of the two pre-columns used for the cold and adsorption trapping have been described previously.[15,16]

Some of the details of gas chromatography and pre-concentration have been reported in previous papers,[17,18] *i.e.*, concentration volumes, 0.005–50 l; detection limits, 0.05–2 p.p.b.; time required for analysis (including the sampling time), less than about 40 min; precision (as coefficient of variation), less than about 10%. This sensitivity and precision are suitable for use in the odour pollution analysis.

The identification of all the odorants was carried out by comparison of the retention times of the sample with those of known standard compounds. The amounts of odorants present were obtained from calibration graphs for known standard compounds.

TABLE I

RESULTS FOR SIX ODORANT GROUPS AT A SEWAGE TREATMENT PLANT

Group	Odorant	Detected concentration, p.p.b.*			R.Th.V.$_n$, p.p.b.†	OU			LogOU			pOU$_m$‡		
		A	B	C		A	B	C	A	B	C	A	B	C
(I), RSR	Hydrogen sulphide	4.6	2.0	4.0	6	—	—	—	—	—	—			
	Methanethiol	6.0	2.0	2.0	0.7	8.6	2.9	2.9	0.93	0.46	0.46			
	Dimethyl sulphide	4.0	2.0	1.0	2	2.0	1.0	—	0.30	0	—	1.23	0.46	0.46
	Dimethyl disulphide	2.0	1.0	1.0	2.8	—	—	—	—	—	—	(1.03)	(0.59)	(0.46)
					OU$_t$	10.6	3.9	2.9						
(III), RCOR	Acetaldehyde	8.6	6.5	6.5	15	—	—	—	—	—	—	0	0	0
(IV), HC§	Toluene	6.5	6.0	6.0	4800	—	—	—	—	—	—	0	0	0
(V), ROH	Methanol	330	244	257	100000¶	—	—	—	—	—	—	0	0	0
	Ethanol	139	104	110	10000¶	—	—	—	—	—	—			
(VI), PhOH	Phenol	1.0	1.0	1.0	59	—	—	—	—	—	—	0	0	0
(VII), RCOOH	Acetic acid	1.6	1.1	1.6	1000¶	—	—	—	—	—	—	0	0	0

* A, Over airing tank; B, over aerobic digestion tank; and C, over first-step precipitation tank.
† Values quoted from reference 12.
‡ Figures in parentheses are pOU$_a$ values.
§ HC represents hydrocarbons.
¶ Values quoted from reference 11.

Reagents

Thirty-three sulphur compounds, 24 lower aliphatic amines, 26 carbonyl compounds, 30 hydrocarbons, 8 lower aliphatic monohydric alcohols, 11 phenols, 8 lower fatty acids and 7 indoles were obtained from Aldrich (Milwaukee, Wisc., USA), Katayama Chemical Industries (Osaka, Japan), Merck (Darmstadt, GFR), PolyScience Corp. (Niles, Ill., USA), Tokyo Kasei Kogyo (Tokyo, Japan) and Wako Pure Chemical Industries (Osaka, Japan). All reagents were of guaranteed or analytical-reagent grade and were used without further purification. Hydrogen sulphide (99.6% minimum) and methanethiol (99.5% minimum) were obtained from Matheson Gas Products, East Rutherford, N.J., USA.

Sensory Test

This test was carried out at the nine sampling sites by sniffing directly the odorous gases. A trained panel of four members of a division of the Air Safety and Environmental Research Center of Aichi Prefecture was used on alternating days for determining the odour quality and odour intensity.

Results and Discussion

Tables I–IX list the values for all the parameters defined earlier in the paper.

In these tables, if the odour recongition threshold values of the odorants being determined are unknown or the OU values are less than 1.0, the odour unit (OU) values of the respective detected odorants are designated as being minus; therefore, the logOU values of the detected odorants are also minus. Further, when the logOU values of all the odorants detected in one odorant group are minus, the pOU_m values of the odorant group is designated as being zero. Therefore, the identification and determination of all of the odorants responsible for odour at a particular odour source are necessary; the determination of precise odour recognition threshold values of most of the odorants that are present at the odour sources are also important. These details are important for further research on odour pollution, its control and the relationship between odorants and odour characteristics from odour sources.

The calculated pOU_m and pOU_a values of the eight odorant groups from the nine odour sources were plotted on polar co-ordinate circular odour charts (Figs. 1 and 2).

TABLE II

RESULTS FOR SEVEN ODORANT GROUPS IN AMBIENT AIR NEAR A FISHMEAL FACTORY

Group	Odorant	Detected concentration, p.p.b.	R.Th.V.$_m$, p.p.b.*	OU	LogOU	pOU_m†
(I), RSR	Hydrogen sulphide	17.9	6	3.0	0.48	1.74 (1.06)
	Methanethiol	3.0	0.7	4.3	0.63	
	Dimethyl sulphide	11.9	2.8	4.3	0.63	
			OU_t	11.6		
(II), R_3N	Trimethylamine	17.8	1	17.8	1.25	1.25 (1.25)
(III), RCOR ..	Acetaldehyde	30.4	15	2.0	0.30	0.30 (0.30)
(IV), HC‡	Benzene	47	4680§	—	—	0
	Toluene	14	4800	—	—	
	Ethylbenzene	5.1	—	—	—	
	p-Xylene	2.9	470§	—	—	
	m-Xylene	6.3	—	—	—	
	o-Xylene	1.7	—	—	—	
(V), ROH	Ethanol	2270	10000§	—	—	0
(VI), PhOH ..	Phenol	1.0	59	—	—	0
(VII), RCOOH ..	Acetic acid	30	1000§	—	—	1.05 (0.86)
	Isobutyric acid	3	1.3	2.3	0.36	
	Butyric acid	4	0.81	4.9	0.69	
			OU_t	7.2		

* Values quoted from reference 12.
† Figures in parentheses are pOU_a values.
‡ HC represents hydrocarbons.
§ Values quoted from reference 11.

TABLE III

RESULTS FOR FIVE ODORANT GROUPS IN EXHAUST GASES FROM A SHELL MOULD PROCESSING PLANT

Group	Odorant	Detected concentration, p.p.b.*			R.Th.V.$_n$, p.p.b.†	OU			LogOU			pOU$_m$‡		
		A	B	C		A	B	C	A	B	C	A	B	C
(I), RSR	Hydrogen sulphide	4.0	1.0	14	6	—	—	2.3	—	—	0.36	—	—	0.36 (0.36)
(III), RCOR	Acetaldehyde	1100	480	1129	15	73.3	32.0	75.3	1.87	1.51	1.88	1.87 (1.87)	1.51 (1.51)	1.88 (1.88)
(IV), HC§	Benzene	667	925	1111	4680¶	—	—	—	—	—	—			
	Toluene	55.6	19.3	596	4800	—	—	—	—	—	—			
	Ethylbenzene	10.4	2.6	33.2	—	—	—	—	—	—	—			
	p-Xylene	8.6	2.1	41.4	470¶	—	—	—	—	—	—	0	0	0
	m-Xylene	20.2	5.2	55.4	—	—	—	—	—	—	—			
	o-Xylene	15.4	6.2	—	—	—	—	—	—	—	—			
	Propylbenzene	1.0	0.5	231	—	—	—	—	—	—	—			
(V), ROH	Methanol	1064	916	44128	100000¶	—	—	—	—	—	—	0	0	0.58
	Ethanol	806	2047	37949	10000¶	—	—	3.8	—	—	0.58			(0.58)
(VI), PhOH	Phenol	750	35.7	2048	59	12.7	—	34.7	1.10	—	1.54	1.10 (1.10)	0	1.54 (1.54)

* A, Core of shell-mould processing; B, end of line casting; and C, shell-mould decomposition workshop.
† Values quoted from reference 12.
‡ Figures in parentheses are pOU$_a$ values.
§ HC represents hydrocarbons.
¶ Values quoted from reference 11.

TABLE IV

RESULTS FOR SIX ODORANT GROUPS AT A NIGHT-SOIL TREATMENT PLANT

Group	Odorant	Detected concentration, p.p.b.* A	B	C	D	R.Th.V.$_n$, p.p.b.†	OU A	B	C	D	LogOU A	B	C	D	pOU$_m$‡ A	B	C	D
(I), RSR	Hydrogen sulphide	64.0	1200	3200	220	6	10.7	200	533	36.7	1.03	2.03	2.73	1.56				
	Methanethiol	1.0	14.0	60.0	4.0	0.7	1.4	20.0	85.7	5.7	0.15	1.03	1.93	0.76	1.58	3.80	6.25	2.95
	Dimethyl sulphide	5.0	11.0	77.0	8.6	2	2.5	5.5	38.5	4.3	0.40	0.74	1.59	0.63	(1.16)	(2.35)	(2.82)	(1.67)
						OU$_t$	14.6	226	657	46.7								
(III), RCOR	Acetaldehyde	8.6	2.5	11.1	4.9	15	—	—	—	—	—	—	—	—	0	0	0	0
(IV), HC§	Benzene	7.6	7.6	13.9	5.1	4680¶	—	—	—	—	—	—	—	—				
	Toluene	18.8	6.4	7.8	5.4	4800	—	—	—	—	—	—	—	—				
	Ethylbenzene	1.1	2.5	5.5	1.1	—	—	—	—	—	—	—	—	—				
	p-Xylene	1.4	1.7	2.9	1.1	470¶	—	—	—	—	—	—	—	—	0	0	0	0
	m-Xylene	3.7	8.5	14.0	11.1	—	—	—	—	—	—	—	—	—				
	o-Xylene	2.5	1.7	2.9	0.4	—	—	—	—	—	—	—	—	—				
(V), ROH	Methanol	16.3	12.2	20.7	12.2	100000¶	—	—	—	—	—	—	—	—				
	Ethanol	39.9	12.3	103	77.4	10000¶	—	—	—	—	—	—	—	—				
	Butan-2-ol	8.8	5.9	—	2.2	220	—	—	—	—	—	—	—	—	0	0	0	0
	Butan-1-ol	1.8	—	—	5.6	—	—	—	—	—	—	—	—	—				
	Pentan-1-ol	16.7	—	—	—	—	—	—	—	—	—	—	—	—				
(VI), PhOH	Phenol	1.0	27.6	97.0	1.0	59	—	—	1.6	—	—	—	0.20	—	0	0	0.20 (0.20)	0
(VII), RCOOH	Butyric acid	0.5	0.5	0.5	0.5	1.3	—	—	—	—	—	—	—	—	0	0	0	0

* A, Near the vacuum dehydration tank for sludge; B, over precipitation tank; C, over bubbling tank for filtrate liquor; and D, near platform for night soil.
† Values quoted from reference 12.
‡ Figures in parentheses are pOU$_a$ values.
§ HC represents hydrocarbons.
¶ Values quoted from reference 11.

TABLE V

RESULTS FOR SEVEN ODORANT GROUPS IN EXHAUST GAS FROM A METAL PAINT DRYER

Group	Odorant	Detected concentration, p.p.b.	R.Th.V.$_n$, p.p.b.*	OU	LogOU	pOU$_m$†
I), RSR	Hydrogen sulphide	1	6	—	—	0.15 (0.15)
	Methanethiol	1	0.7	1.4	0.15	
	Dimethyl sulphide	1	2	—	—	
(III), RCOR ..	Acetaldehyde	21200	15	1413	3.15	7.32 (3.30)
	Propionaldehyde	3160	—	—	—	
	Acrolein	2670	100	26.7	1.43	
	Acetone	4100	100000‡	—	—	
	Butyraldehyde	2030	3.7	549	2.74	
	Valeraldehyde	12000	—	—	—	
			OU$_t$	1989	—	
(IV), HC§	Benzene	50	4680‡	—	—	0
	Toluene	20	4800	—	—	
	Ethylbenzene	12.2	—	—	—	
(V), ROH	Methanol	15682	100000‡	—	—	0.70 (0.70)
	Ethanol	1846	10000‡	—	—	
	Propan-2-ol	5050	—	—	—	
	Propan-1-ol	829	—	—	—	
	Butan-2-ol	1091	220	5.0	0.70	
	Butan-1-ol	547	—	—	—	
	Pentan-1-ol	1336	—	—	—	
(VI), PhOH ..	Phenol	38.8	59	—	—	0.28 (0.28)
	o-Cresol	11.3	6.1	1.9	0.28	
	p-Ethylphenol	23.1	—	—	—	
	2,6-Xylenol	1.3	—	—	—	
(VII), RCOOH	Propionic acid	1546	8.4	184	2.26	4.90 (2.80)
	Butyric acid	353	0.8	441	2.64	
	Valeric acid	62	—	—	—	
			OU$_t$	625		
(VIII), Indoles ..	Indole	14.9	0.05¶	298	2.47	2.47 (2.47)

* Values quoted from reference 12.
† Figures in parentheses are pOU$_a$ values.
‡ Values quoted from reference 11.
§ HC represents hydrocarbons.
¶ Value quoted from reference 13.

Figs. 1 and 2 indicate that the shapes and sizes of the circular odour charts could relate the quality and the intensity of the odour perception to the odours from some processes or factories belonging to the nine odour sources. The relationship between the odour perception (odour quality and intensity) and the pOU$_m$, pOU$_a$ and logOU values of the nine odour sources are listed in Table X. The large values were due to the odours or odorant group that contributed most to the intensity of the odour.

The frequencies in each odorant group of the odorants that give a large contribution to the odour perception at the nine odour sources are as follows: group (I), sulphur compounds were present at seven odour sources; group (II), lower aliphatic amines were present at three odour sources; group (III), carbonyl compounds were present at four odour sources; group (IV), hydrocarbons were zero; group (V), lower aliphatic monohydric alcohols were zero; group (VI), phenols were present at one odour source; group (VII), lower fatty acids were present at four odour sources; and group (VIII), indoles were present at three odour sources. As shown in Table X, the relationship between the odour intensity of the foetid gases from the nine odour sources and the pOU$_m$, pOU$_a$ and logOU values of the eight odorant groups were as follows: (*i*) for a moderate odour intensity, there will be around 1 odorant group with values for pOU$_m$ and pOU$_a$ of about 1, including 1 odorant that has a logOU value of about 1; (*ii*) for a strong odour intensity, there will be around 1–2 odorant groups with pOU$_m$ values of about 2–6 and pOU$_a$ values of about 1–3, including 1 or 2 odorants with logOU values of about 1–2; and (*iii*)

TABLE VI

RESULTS FOR FIVE ODORANT GROUPS IN EXHAUST GAS FROM THREE KRAFT PULP RECOVERY BOILERS

Group	Odorant	Detected concentration, p.p.b.*			R.Th.V.$_{n}$,	OU			LogOU			pOU$_{m}$‡		
		A	B	C	p.p.b.†	A	B	C	A	B	C	A	B	C
(I), RSR	Hydrogen sulphide	530	22.9	186	6	88.3	3.8	31.0	1.95	0.58	1.49			
	Methanethiol	194	131	167	0.7	277	187	239	2.44	2.27	2.38			
	Dimethyl sulphide	227	96.2	539	2	114	48.1	270	2.06	1.68	2.43	9.18	6.94	9.31
	Dimethyl disulphide	120	61.0	225	2.8	42.9	21.8	80.4	1.63	1.34	1.91	(2.73)	(2.43)	(2.80)
	Dimethyl trisulphide	17.8	16.5	17.8	1.4§	12.7	11.8	12.7	1.10	1.07	1.10			
					OU_t	535	273	633						
(III), RCOR	Acetaldehyde	168	269	222	15	11.2	17.9	14.8	1.05	1.25	1.17	1.05	1.25	1.17
	Acetone	1254	1378	616	100000¶	—	—	—	—	—	—	(1.05)	(1.25)	(1.17)
(IV), HC‖	Benzene	38.3	13.3	38.9	4680¶	—	—	—	—	—	—			
	Toluene	42.2	93.4	18.7	4800	—	—	—	—	—	—			
	Ethylbenzene	230	43.8	13.9	—	—	—	—	—	—	—			
	p-Xylene	—	7.8	8.3	470¶	—	—	—	—	—	—			
	m-Xylene	—	18.8	19.0	—	—	—	—	—	—	—	0	0	0
	o-Xylene	—	12.4	9.7	—	—	—	—	—	—	—			
	Styrene	—	—	5.5	170	—	—	—	—	—	—			
	Propylbenzene	—	—	7.5	—	—	—	—	—	—	—			
(V), ROH	Methanol	3970	8810	14500	100000¶	—	—	—	—	—	—			
	Ethanol	918	1853	2140	10000¶	—	—	—	—	—	—	0	0.08	0.08
	Propan-2-ol	141	167	799	—	—	—	—	—	—	—		(0.08)	(0.08)
	Butan-2-ol	70.3	259	260	220	—	1.2	1.2	—	0.08	0.08			
(VI); PhOH	Phenol	4.5	414	367	59	—	7.0	6.2	—	0.85	0.79	0	0.85	0.79
													(0.85)	(0.79)
(VII), RCOOH	Acetic acid	—	272	—	1000¶	—	—	—	—	—	—			
	Isobutyric acid	—	7.2	—	2.7	—	2.7	—	—	0.43	—	0	0.43	0
	Valeric acid	11.4	—	—	—	—	—	—	—	—	—		(0.43)	

* A, B and C are three different recovery boilers.
† Values quoted from reference 12.
‡ Figures in parentheses are pOU_a values.
§ Values quoted from reference 14.
¶ Values quoted from reference 11.
‖ HC represents hydrocarbons.

TABLE VII

RESULTS FOR SEVEN ODORANT GROUPS IN HEADSPACE OVER KRAFT PULP WASTEWATER

Group	Odorant	Detected concentration, p.p.b.	R.Th.V.$_n$, p.p.b.	OU	LogOU	pOU$_m$†
(I), RSR	Hydrogen sulphide	357	6	59.5	1.77	
	Carbon disulphide	282	210‡	1.3	0.11	
	Methanethiol	100	0.7	143	2.16	
	Dimethyl sulphide	7310	2	3655	3.56	12.91 (3.74)
	Dimethyl disulphide	4140	2.8	1479	3.17	
	Dimethyl trisulphide	193	1.4§	138	2.14	
			OU$_t$	5476		
(III), RCOR ..	Acetaldehyde	159	15	10.6	1.03	1.03 (1.03)
(IV), HC¶ ..	Isoprene	2911	—	—	—	
	α-Pinene	1618	—	—	—	
	β-Pinene	55.2	—	—	—	0
	Toluene	6.5	4800	—	—	
	o-Xylene	326	—	—	—	
(V), ROH	Methanol	1225	100000‡	—	—	0
	Ethanol	3152	10000‡	—	—	
(VI), PhOH ..	Phenol	16.2	59	—	—	0
(VII), RCOOH ..	Acetic acid	122	1000‡	—	—	
	Propionic acid	164	8.4	19.5	1.29	1.29 (1.29)
	Valeric acid	23.8	—	—	—	
(VIII), Indoles ..	1,2-Dimethylindole	0.93	—	—	—	1.27 (1.27)
	Indole	0.94	0.05‖	18.8	1.27	

* Values quoted from reference 12.
† Figures in parentheses are pOU$_a$ values.
‡ Values quoted from reference 11.
§ Values quoted from reference 14.
¶ HC represents hydrocarbons.
‖ Values quoted from reference 13.

for a very strong odour intensity there will be from 1 to several odorant groups with pOU$_m$ values of about 6–15 and pOU$_a$ values of about 2.5–4, including 1 to several odorants with logOU values of about 2–3.

Although in practice many odorants are responsible for odour characteristics, it is important to determine the relationship between odour characteristics (quality and intensity) and analytical data for the odorants responsible for the odours discharged from several odour sources, and to recognise the magnitude of the contribution of each odorant group and the detected odorants to the odour perception. The terms pOU$_m$ and pOU$_a$ in this study represent the total effect of multiplying and adding the odour units of each detected odorant responsible for the odours discharged at the nine odour sources.

As listed in Table X, for odours of moderate intensity the pOU$_m$ values were in reasonable agreement with the pOU$_a$ values. However, for strong and very strong odours, which were often unpleasant, the differences between the pOU$_m$ and pOU$_a$ values were significant. These results are important for evaluating the additive and synergistic effects of odorants on odour intensity or the variation of odour quality of mixtures of odorants.

For example, in the sensory tests on the exhaust gases from two poultry manure dryers (A and B) the odour perceptions were stronger, more rancid, more putrid and smelt more of rotten eggs and cabbage than ammoniacal. As shown in Fig. 2, this fact is demonstrated better in the plot of the pOU$_m$ values rather than of the pOU$_a$ values, because the values for each of the pOU$_a$ values of the odorant groups (I), (II), (III), (VII) and (VIII) were almost the same, which does not suggest strong, unpleasant odours. Hence, as listed in Table VIII, the maximum OU values of odorants in these groups were as follows: (I) methanethiol, 1714 for A, 729 for B; (II) ammonia, 333 for A, 283 for B; (III) acetaldehyde, 298 for A, 277 for B; (VII) isovaleric acid 5889 for A, butyric acid 683 for B; and (VIII) indole 49.8 for A, 152 for B. Therefore, it is thought that from the detected odorants the maximum contribution to odour perception is due to isovaleric acid for dryer A and methanethiol for dryer B.

TABLE VIII

RESULTS FOR EIGHT ODORANT GROUPS IN EXHAUST GAS FROM TWO POULTRY MANURE DRYERS

Group	Odorant	Detected concentration, p.p.b.* A	Detected concentration, p.p.b.* B	R.Th.V.$_n$, p.p.b.†	OU A	OU B	LogOU A	LogOU B	pOU$_m$‡ A	pOU$_m$‡ B
(I), RSR	Hydrogen sulphide	400	200	6	66.7	33.3	1.82	1.52		
	Methanethiol	1200	510	0.7	1714	729	3.23	2.86		
	Dimethyl sulphide	100	88.0	2	50.0	44.0	1.70	1.64		
	Ethanethiol	50.0	—	0.3	167	—	2.22	—	10.69	7.13
	Dimethyl trisulphide	74.1	0.4	1.4§	53.0	—	1.72	—	(3.31)	(2.91)
	Sulphur dioxide	—	6000	470	—	12.8	—	1.11		
				OU$_t$	2051	819				
(II), R_3N	Trimethylamine	20.0	144	1	20.0	144	1.30	2.16		
	Ethylamine	100	150	6000	—	—	—	—	3.82	4.61
	Ammonia	200000	170000	600	333	283	2.52	2.45	(2.55)	(2.63)
				OU$_t$	353	427				
(III), RCOR	Acetaldehyde	4470	4150	15	298	277	2.47	2.44		
	Propionaldehyde	440	305	—	—	—	—	—		
	Acrolein	1830	1360	100	18.3	13.6	1.26	1.13		
	Acetone	740	597	100000¶	—	—	—	—	5.62	4.78
	Isobutyraldehyde	800	160	—	—	—	—	—	(2.60)	(2.49)
	Butyraldehyde	290	60	3.7	78.4	16.2	1.89	1.21		
	Ethyl methyl ketone	260	8	10000¶	—	—	—	—		
	Isovaleraldehyde	1980	165	—	—	—	—	—		
				OU$_t$	395	307				
(IV), HC‖	Benzene	250	1274	4680¶	—	—	—	—	0	0
	Toluene	120	50.5	4800	—	—	—	—		
(V), ROH	Methanol	520	1328	100000¶	—	—	—	—	0	0
	Ethanol	1070	1420	10000¶	—	—	—	—		
(VI), PhOH	Phenol	151	116	59	2.6	2.0	0.42	0.30	0.42	0.30
	o-Ethylphenol	42	—	—	—	—	—	—	(0.42)	(0.30)
(VII), RCOOH	Acetic acid	—	1696	1000¶	—	1.7	—	0.23		
	Propionic acid	—	1190	8.4	—	142	—	2.15		
	Isobutyric acid	7400	399	1.3	5692	307	3.76	2.49		
	Butyric acid	1900	546	0.81	2375	683	3.38	2.83	10.91	9.66
	Isovaleric acid	15900	249	2.7	5889	92.2	3.77	1.96	(4.15)	(3.09)
	Valeric acid	200	37.4	—	—	—	—	—		
				OU$_t$	13956	1226				

TABLE VIII—continued

Group	Odorant	Detected concentration, p.p.b.* A	Detected concentration, p.p.b.* B	R.Th.V.$_n$, p.p.b.†	OU A	OU B	LogOU A	LogOU B	pOU$_m$‡ A	pOU$_m$‡ B
(VIII), Indoles	1,2-Dimethylindole	1.04	8.95	—	—	—	—	—		
	Indole	2.49	7.60	0.05**	49.8	152	1.70	2.18		
	2-Methylindole	0.45	—	—	—	—	—	—	1.70	2.18
	3-Methylindole	1.15	0.20	—	—	—	—	—	(1.70)	(2.18)
	5-Methylindole	0.55	—	—	—	—	—	—		
	2,3-Dimethylindole	2.55	—	—	—	—	—	—		

* A and B are two different poultry manure dryers.
† Values quoted from reference 12.
‡ Figures in parentheses are pOU$_a$ values.
§ Values quoted from reference 14.
¶ Values quoted from reference 11.
|| HC represents hydrocarbons.
** Values quoted from reference 13.

TABLE IX

RESULTS FOR EIGHT ODORANT GROUPS IN EXHAUST GAS FROM A CORN STARCH MANUFACTURING WORKS

Group	Odorant	Detected concentration, p.p.b.	R.Th.V.$_n$, p.p.b.*	OU	LogOU	pOU$_m$†
(I), RSR	Hydrogen sulphide	2700	6	450	2.65	
	Carbon disulphide	3.1	210‡	—	—	
	Methanethiol	1300	0.7	1857	3.27	
	Ethanethiol	1500	0.3	5000	3.70	15.54 (3.99)
	Dimethyl sulphide	1100	2.8	393	2.59	
	Sulphur dioxide	1000000	470‡	2128	3.33	
			OU$_t$	9828		
(II), R_3N	Trimethylamine	830	1	830	2.92	
	Ammonia	100000	600	167	2.22	5.14 (3.00)
			OU$_t$	997		

TABLE IX—continued

Group	Odorant	Detected concentration, p.p.b.	R.Th.V.$_n$, p.p.b.*	OU	LogOU	pOU$_m$†
(III), RCOR ..	Acetaldehyde	5320	15	355	2.55	
	Acrolein	770	100	7.7	0.89	
	Acetone	770	100000‡	—	—	3.44 (2.56)
	Ethyl methyl ketone	40	10000‡	—	—	
	Isovaleraldehyde	3020	—	—	—	
			OU$_t$	363	—	
(IV), HC§	Toluene	71	4800	—	—	0
(V), ROH	Methanol	3840	100000‡	—	—	
	Ethanol	32500	10000‡	3.3	0.52	
	Propan-2-ol	16	—	—	—	0.52 (0.52)
	Propan-1-ol	16	—	—	—	
	Butan-2-ol	43	220	—	—	
	Butan-1-ol	43	—	—	—	
(VI), PhOH ..	Phenol	67	59	1.1	0.04	
	o-Cresol	28	6.1	4.6	0.66	0.70 (0.76)
			OU$_t$	5.7		
(VII), RCOOH ..	Acetic acid	2000	1000‡	2.0	0.30	
	Propionic acid	32.0	8.4	3.8	0.58	
	Isobutyric acid	33.9	1.3	26.1	1.42	
	Butyric acid	56.6	0.81	69.9	1.84	5.06 (2.04)
	Isovaleric acid	21.6	2.6	8.3	0.92	
	Valeric acid	182.2	—	—	—	
			OU$_t$	110		
(VIII), Indoles ..	Indole	19.3	0.05¶	386	2.59	2.59 (2.59)
	5-Methylindole	11.2	—	—	—	

* Values quoted from reference 12.
† Figures in parentheses are pOU$_a$ values.
‡ Values quoted from reference 11.
§ HC represents hydrocarbons.
¶ Value quoted from reference 13.

TABLE X

RELATIONSHIP BETWEEN ODOUR CHARACTERISTICS AND pOU_m, pOU_a AND LogOU VALUES FOR NINE ODOUR SOURCES

For identification of A, B, C and D, see footnotes of respective Tables.

Odour source	Odour quality and intensity	Odorant groups: $pOU_m > 1$*	Odorant groups: $pOU_m = 0 \sim 1$*	Odorant groups: pOU_m and $pOU_a = 0$	LogOU of odorants
Air over tanks of sewage water treatment plant	Sulphur-like, sewage, sludge Moderate	(I)RSR: A, 1.23 (1.03); B, 0.46 (0.59); C, 0.46 (0.46)	—	(III)RCOR (IV)HC (V)ROH (VI)PhOH (VII)RCOOH	Methanethiol: A, 0.93; B, 0.46; C, 0.46 Dimethyl sulphide: A, 0.30; B, 0; C, —
Ambient air near fishmeal factory	Fishy, faecal, putrid, rancid Moderate to strong	(I)RSR: 1.74 (1.06) (II)R_3N: 1.25 (1.25) (VII)RCOOH: 1.05 (0.86)	(III)RCOR: 0.30 (0.30)	(IV)HC (V)ROH (VI)PhOH	Trimethylamine: 1.25 Butyric acid: 0.69 Dimethyl disulphide: 0.63 Methanethiol: 0.63 Isobutyric acid: 0.36 Acetaldehyde: 0.30
Exhaust gas from shell-mould processing	Phenolic Moderate	(III)RCOR: A, 1.87 (1.87); B, 1.51 (1.51); C, 1.88 (1.88) (VI)PhOH: A, 1.10 (1.10); B, 0; C, 1.54 (1.54)	(V)ROH: A and B, 0; C, 0.58 (0.58) (I)RSR; A and B, 0; C, 0.36 (0.36)	(IV)HC	Acetaldehyde: A, 1.87; B, 1.51; C, 1.88 Phenol: A, 1.10; B, 0; C, 1.54
Exhaust gas from night-soil treatment plant	Sulphur-like, ammoniacal Strong	(I)RSR: A, 1.58 (1.16); B, 3.80 (2.35); C, 6.25 (2.82); D, 2.95 (1.67)	(VI)PhOH: A and B, 0; C; 0.20 (0.20); D, 0	(III)RCOR (IV)HC (V)ROH (VII)RCOOH	Hydrogen sulphide: A, 1.03; B, 2.03; C, 2.73, D, 1.56 Methonethird A, 0.15; B, 1.03; C, 1.93; D, 0.76 Dimethyl sulphide A, 0.40; B, 0.74; C, 1.59; D, 0.63
Exhaust gas from metal paint dryer	Pungent, putrid Strong	(III)RCOR: 7.32 (3.30) (VII)RCOOH: 4.90 (2.80) (VIII)Indoles: 2.47 (2.47)	(V)ROH: 0.70 (0.70) (VI)PhOH: 0.28 (0.28) (I)RSR: 0.15 (0.15)	(IV)HC	Acetaldehyde: 3.15 Butyraldehyde: 2.74 Butyric acid: 2.64 Indole: 2.47 Propionic acid: 2.26 Acrolein: 1.43
Exhaust gas from kraft pulp recovery boilers	Sulphur-like, pulpy Very strong	(I)RSR: A, 9.18 (2.73); B, 6.94 (2.43); C, 9.31 (2.81)	(VI)PhOH: A, 0; B, 0.85 (0.85); C, 0.79 (0.79)	(III)RCOR (IV)HC (V)ROH	Dimethyl sulphide: A, 2.06; B, 1.68; C, 2.43 Methanethiol: A, 2.44; B, 2.27; C, 2.38 Dimethyl disulphide: A, 1.63; B, 1.34; C, 1.91 Hydrogen sulphide: A, 1.95; B, 0.58; C, 1.49
Headspace over kraft pulp wastewater	Sulphur-like, putrid, pulpy Very strong	(I)RSR: 12.91 (3.74)	(VII)RCOOH: 1.29 (1.29) (VIII)Indoles: 1.27 (1.27) (III)RCOR: 1.03 (1.03)	(IV)HC (V)ROH (VI)PhOH	Dimethyl sulphide: 3.56 Dimethyl disulphide: 3.17 Methanethiol: 2.16 Dimethyl trisulphide: 2.13 Hydrogen sulphide: 1.77 Propionic acid: 1.29 Indole: 1.27

TABLE X—continued

Odour source	Odour quality and intensity	Odorant groups: $pOU_m > 1$*	$pOU_m = 0 \sim 1$*	pOU_m and $pOU_a = 0$	LogOU of odorants
Exhaust gas from poultry manure dryers	Ammoniacal, rancid, like rotten eggs and rotten cabbage, faecal, putrid Very strong	(VII)RCOOH: A, 10.91 (4.15); B, 9.66 (3.09) (I)RSR: A, 10.69 (3.31); B, 7.13 (2.91) (III)RCOR: A, 5.62 (2.60); B, 4.78 (2.49) (II)R_3N: A, 3.82 (2.55); B, 4.61 (2.63) (VIII)Indoles: A, 1.70 (1.70); B, 2.18 (2.18)	(VI)PhOH: A, 0.42 (0.42); B, 0.30 (0.30)	(IV)HC (V)ROH	Isovaleric acid: A, 3.77; B, 1.96 Isobutyric acid: A, 3.76; B, 2.49 Butyric acid: A, 3.38; B. 2.83 Methanethiol: A, 3.23; B, 2.86 Ammonia: A, 2.52; B, 2.45 Acetaldehyde: A, 2.47; B, 2.44 Butyraldehyde: A, 1.89; B, 1.21 Hydrogen sulphide: A, 1.82; B, 1.52 Dimethyl trisulphide: A, 1.72; B, — Dimethyl sulphide: A, 1.70; B, 1.64 Indole: A, 1.70; B, 2.18 Trimethylamine: A, 1.30; B, 2.16 Acrolein: A, 1.26; B, 1.13
Exhaust gas from corn starch manufacturing works	Sulphur-like, pungent, rancid, sour, Very strong	(I)RSR: 15.54 (3.99) (II)R_3N: 5.14 (3.00) (VII)RCOOH: 5.06 (2.04) (III)RCOR: 3.44 (2.56) (VIII) Indoles: 2.59 (2.59)	(VI)PhOH: 0.70 (0.70) (V)ROH: 0.52 (0.52)	(IV)HC	Ethanethiol: 3.70 Sulphur dioxide: 3.33 Methanethiol: 3.27 Trimethylamine: 2.92 Hydrogen sulphide: 2.65 Dimethyl disulphide: 2.59 Indole: 2.59 Acetaldehyde: 2.55 Ammonia: 2.22 Butyric acid: 1.84 Isobutyric acid: 1.42

* Figures in parentheses are pOU_a values

Further, in the sensory test on the exhaust gas from the corn-starch manufacturing works, odour perceptions were stronger, sulphur like, pungent, rancid and sour odours, which are based on the odorant groups (I), (III) and (VII). However, the magnitudes of each pOU_a value in these odorant groups were the same as those of odorant groups (II) and (VIII). This fact cannot be explained simply by saying that the additive effect of the odorants is responsible for the odour. As listed in Table IX, the maximum OU values in these groups were as follows: (I) ethanethiol 5000; (II) trimethylamine 830; (III) acetaldehyde 355; (VII) butyric acid 69.9; and (VIII) indole 386. Therefore, it is thought that ethanethiol gives the maximum contribution to the odour perception for the odour from the corn starch manufacturing works.

As described above, the existence of a synergistic effect may be demonstrated in odorant groups (I) and (VII) for the exhaust gases from poultry manure dryers and in odorant group (I) for the exhaust gases from the corn starch manufacturing works.

The pOU_m term is important in order to emphasise the contribution to odour perception of each odorant group. Further, in the programming necessary for the control of odour pollution in many areas it may be possible to recognise the magnitude of the contribution to the odour perception of each odorant group by using a simple graphic illustration of the complex odorant mixtures.

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Separation and Identification of Aminocarboxylic Acid Sequestrants by High-performance Liquid Chromatography

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A simple method has been developed for the separation by high-performance liquid chromatography of some common aminocarboxylic acid sequestrants as their copper complexes. The copper complex is formed *in situ* by using a copper salt solution as the eluate. Detection using visible light at 760 nm eliminates interference from ultraviolet absorbing compounds often present in mixtures containing sequestrants.

Keywords: High-performance liquid chromatography; aminocarboxylic acids; sequestrants; copper complexes

The extensive use of aminocarboxylic acid sequestrants, such as ethylenediaminetetraacetic acid (EDTA), particularly in detergent products, gives rise to the need for a method to distinguish between the different sequestrants, especially if more than one is present in the mixture. The normal titration procedures previously described[1–3] that depend upon the chelating properties of the aminocarboxylic acids do not distinguish between them and furthermore are not applicable to *NN*-(dihydroxyethyl)glycine (DHEG).

Several workers[4–8] have described gas-chromatographic methods for the separation of aminocarboxylic acids but all of these require isolation and conversion into a volatile derivative.

Iguchi *et al.*[9] described a method for the determination of EDTA by utilising the ultraviolet absorption of its copper complex at 248 nm. Perfetti and Warner[10] have determined EDTA in foodstuffs by means of high-performance liquid chromatography of its copper complex and using ultraviolet absorption at 254 nm for detection. Both of these methods require careful separation of the sequestrant from other substances that absorb ultraviolet light at these wavelengths. Furthermore these papers refer only to EDTA although they could probably be adapted for other sequestrants.

The method described here can be used to identify aminocarboxylic acids without prior derivatisation or separation from most other compounds normally present in detergent compositions. It had been observed in our laboratory that the addition of a sequestrant to a coloured metallic ion solution often produced an intensification of the colour and that this was particularly noticeable with copper(II) salts. This intensification of colour was used for detection in the high-performance liquid chromatographic method developed.

Experimental

The following sequestrants were investigated: *NN*-(dihydroxyethyl)glycine (DHEG); nitrilotriacetic acid (NTA); ethylenediaminetetraacetic acid (EDTA); diethylenetriaminepentaacetic acid (DTPA); and *N*-hydroxyethylethylenediaminetriacetic acid (HEDTA).

The equipment used was a Varian 5000 high-performance liquid chromatograph with a Varichrom variable wavelength detector: wavelength, 760 nm; band width, 16 nm; time constant, slow; column, 250 × 4.6 mm packed with 5-μm Spherisorb ODS; and flow-rate, 1 ml min^{-1}.

The eluting solution was 0.2 M in ammonium chloride (AnalaR), 0.01 M in copper(II) chloride (AnalaR) and 0.05 M in benzyltrimethylammonium chloride (BTAC) prepared using HPLC-grade water and adjusted to pH 3.5–4.0 with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide solution.

Procedure

The sequestrant solutions were 0.02 M and adjusted to pH 3–4. A 10-μl sample was injected by means of a fixed volume loop injector and eluted at 1.0 ml min^{-1}.

Results and Discussion

Fig. 1 is a typical chromatogram showing that a good separation of the five sequestrants was obtained in about 8 min. As tests on single sequestrants showed that they had different response factors the mixture actually used to produce Fig. 1 had the following composition: DHEG, 0.02 M; NTA, 0.025 M; DTPA, 0.04 M; HEDTA, 0.015 M; and EDTA, 0.025 M. In earlier experiments other counter ions had been used, namely tetramethylammonium chloride, tetrabutylammonium chloride and dodecyltrimethylammonium bromide, but none of these gave as good a separation as benzyltrimethylammonium chloride.

A series of experiments was carried out to determine whether the method could be used to quantify the sequestrants. In the absence of an integrator it was found that reasonably satisfactory quantitative determinations could be made using peak-height measurements. Quadruplicate injections of solutions of sequestrants of differing concentrations were made and peak heights measured. The absorbance factor of the detector was varied to give peaks of reasonable height and these were then corrected to a standard absorbance factor. Tables I and II show typical results obtained for NTA and DHEG.

Graphs of corrected peak height against concentration for both sequestrants are straight lines. The method can thus be used for both quantitative determination and identification. The limit of detection using a 10-μl sample would appear to be about a 0.002 M solution of sequestrant.

A number of tests were carried out to evaluate the effect of various substances that may be found in detergent compositions. In each instance a preliminary experiment was carried out in order to see if there would be any undesirable interaction between the detergent ingredient and the eluting solution that could lead to damage to the column packing. The results of these tests and the action that was taken to overcome the problems that arose are detailed below.

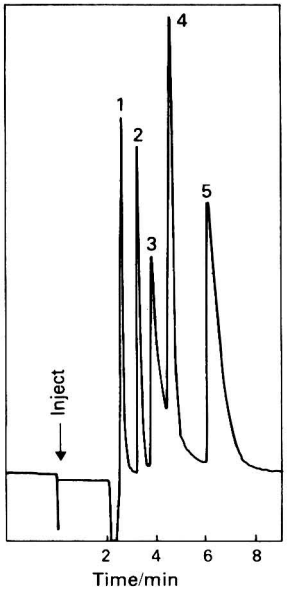

Fig. 1. Typical chromatogram showing separation of five sequestrants: 1, DHEG; 2, NTA; 3, DPTA; 4, HEDTA; and 5, EDTA.

TABLE I

PEAK HEIGHTS FOR SAMPLES OF NTA

The volume of each sample was 10 μl.

NTA concentration/ M	Absorbance range	Peak heights, arbitrary units				Average peak height, arbitrary units	Corrected to an absorbance of 0.2
0.00333	0.02	37.5	37.5	38.0	36.0	37.3	3.7
0.00333	0.05	16.0	17.0	16.5	16.5	16.5	4.1
0.00667	0.05	37.5	39.0	38.0	40.0	38.6	9.7
0.00667	0.10	20.5	19.5	19.0	20.0	19.8	9.9
0.0133	0.1	49.0	49.0	48.0	48.0	48.5	24.3
0.0133	0.2	25.0	24.5	24.5	24.0	24.5	24.5
0.0200	0.2	38.0	39.0	38.5	38.5	38.5	38.5
0.0267	0.2	52.0	52.5	52.0	53.0	52.4	52.4
0.0333	0.2	64.0	65.5	65.0	67.0	65.4	65.4

TABLE II

PEAK HEIGHTS FOR SAMPLES OF DHEG

The volume of each sample was 10 μl.

DHEG concentration/ M	Absorbance range	Peak heights, arbitrary units				Average peak height, arbitrary units	Corrected to an absorbance of 0.2
0.00333	0.05	22.0	23.0	22.5	22.5	22.5	5.6
0.00667	0.05	41.0	40.0	37.5	39.0	39.4	9.9
0.00667	0.1	22.0	23.0	23.0	22.5	22.6	11.3
0.0133	0.1	45.5	46.0	46.5	46.0	46.0	23.0
0.0200	0.1	70.5	69.5	69.5	69.5	69.8	34.9
0.0200	0.2	35.0	35.0	35.5	34.5	35.0	35.0
0.0267	0.2	50.0	49.5	48.0	48.0	48.7	48.7
0.0333	0.2	61.5	61.5	61.5	61.0	61.4	61.4

Non-ionic Surfactants

Neither nonylphenol- nor alcohol-based ethylene oxide condensates had any effect on the determination.

Anionic Surfactants

Both sodium dodecylbenzenesulphonate and sodium lauryl ether sulphate caused slight increases in the retention times.

Cationic Surfactants

Benzalkonium chloride [*N*-alkyl-*N*-benzyl-*NN*-dimethylammonium chloride (where alkyl = C_8–C_{18})] and amine - ethylene oxide condensates caused slight reductions in retention times.

Fatty Acid Soaps

Separation of free fatty acids occurs at pH 3.5–4. These could then be easily removed by extraction with light petroleum (b.p. 40–60 °C) or centrifuging.

Inorganic Salts

Sodium chloride, nitrate, borate and orthophosphate caused no interferences. Penta-sodium triphosphate and pyrophosphate produced a narrowing of the peaks and shorter retention times. Sodium nitrite and carbonate produced gas bubbles at pH 3.5–4 but if the acidified solutions were boiled before injection there was no interference. Sodium silicates precipitated silica at pH 3.5–4 but this could be removed by filtration and caused no other interference. Sodium acetate produced a peak with a retention time similar to that of DTPA but if the acidified solution was boiled to steam distil off the acetic acid there was no further interference.

As these results show that in some detergent compositions there could be ingredients that affect the retention time a procedure has been devised to overcome any doubt as to the identity of an unidentified sequestrant. A solution of the sample is prepared to have a sequestrant concentration of about 0.01–0.03 M. An aliquot of this solution is mixed with an equal volume of the five-sequestrant mixture referred to earlier. A similar 1 + 1 dilution of the five-sequestrant mixture is made with distilled water. The chromatograms produced from these two solutions are compared. If one or more of the peaks in the chromatogram from the solution containing the sample are bigger than those in the chromatogram of the diluted five-sequestrant mixture then this identifies the unknown sequestrant or sequestrants in the sample.

This procedure has been used in our laboratory to confirm the presence of sequestrants in our own products and to identify sequestrants in unknown mixtures. With an integrator, it should be possible to measure the increase in peak size and to thus quantify the sequestrant as well as identify it and it is proposed to carry out further work on the use of this method to quantify the sequestrants.

The sequestrants examined in this work are the most common commercially used materials and although other sequestrants are sometimes, but rarely, used, it is unlikely that they would have retention times identical with those reported here. If therefore a sample contained a sequestrant other than those so far tested, it would produce an additional peak or at least a fused peak or shoulder. It was mentioned earlier that acetic acid could be detected by this chromatographic method and tests have shown that acetic, propionic and butyric acids can be separated with retention times of about 4, 6.5 and 10 min, respectively. However, the peaks become wider as the relative molecular mass of the acid increases but the eluting solution could no doubt be modified if it was considered that the method would be of value in the analysis of short chain carboxylic acids. The response factors of carboxylic acids are only about one tenth of those of the sequestrants at equimolar concentrations.

Similarly, it has been found that a number of naturally occurring amino acids can be detected using the same eluting and detection method. The amino acids tested and their retention times are listed in Table III.

TABLE III

RETENTION TIMES OF SOME NATURALLY OCCURRING AMINO ACIDS

Amino acid	Retention time/min
DL-Alanine	2.5
DL-2-Aminobutyric acid	2.6
DL-Aspartic acid	2.8
L-Cysteine hydrochloride	4.5
L-Glutamic acid	2.8
L-Histidine	2.5
L-Hydroxyproline	2.7
L-Lencine	3.4
DL-Methionine	3.2
DL-β-phenylalanine	5.8
L-Proline	2.9
L-Tyrosine	4.0
DL-Valine	3.0

It would appear therefore that the colour change of inorganic salt solutions could be of use as a detection method for a number of high-performance liquid chromatographic separations where other methods of detection fail or derivatisation is necessary to facilitate detection Work is in progress to apply this method to other types of sequestrants, such as gluconates and phosphonates.

The author thanks Dr. J. D. Dance (Technical Director) and the Directors of Applied Chemicals Limited for permission to publish this paper.

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Analytical Methods Committee

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

Determination of Pyrimethamine in Animal Feeds

Keywords: *Pyrimethamine determination*; *animal feeds*; *gas - liquid chromatography*

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 until March 1979), Mr. R. S. Hatfull (Chairman from March 1979), Mr. R. J. Anderson, Mr. A. G. Croft, Mr. R. J. Davies (from March 1979), Mr. R. Fawcett (until March 1979), Mr. J. R. Harris, Mr. G. Kitson, Mr. G. R. Merson (from October 1979), Mr. D. H. Mitchell, Mr. J. A. Stubbles, with (the late) Dr. N. W. Hanson (until August 1979) and Mr. J. J. Wilson (from September 1979) as Secretaries. Some of these members are also members of the Medicinal Additives in Animal Feeds Sub-Committee (B), which also participated in the discussions on this method.

Introduction

Pyrimethamine [5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diyldiamine] is a drug used as a coccidiostat in rabbit and poultry feeds, normally in conjunction with sulphaquinoxaline. In 1977 the Analytical Methods Committee (AMC) published a report from its Medicinal Additives in Animal Feeds Sub-Committee (B) in which they described a procedure for the determination of pyrimethamine by spectrophotometry.[1] This method used benzene as a solvent, which was known to be undesirable, but no suitable alternative solvent was known. The presence of grassmeal in the feed caused interference and the method was rejected by the EEC Group of Experts on these grounds. However, the AMC decided to publish the method as there was at that time no official method for the determination of pyrimethamine and the only known uses of the drug in the UK were in feeds that did not contain grassmeal.

A procedure for the determination of pyrimethamine by gas - liquid chromatography, which was not subject to interference from grassmeal, was published by Harris *et al.*[2] and this gave satisfactory results when tried by some members of the EEC Group of Experts. The Group of Experts under the Chairmanship of Dr. S. Dormal van den Bruel then decided to carry out a full collaborative trial of this method and as they considered it desirable to have as many sets of results as possible they suggested that other laboratories in the UK should be invited to participate. Some members of both the A and B Sub-Committees took part in the exercise and all the results obtained are given in this report.

Experimental

The method employed was that described by Harris *et al.*[2] Some preliminary trials were carried out on samples of rabbit and poultry feeds containing 5 mg kg^{-1} of pyrimethamine and recoveries of 73–97% were reported. A sample of poultry feed containing 4.9 mg kg^{-1} was prepared by addition of a pre-mix of known pyrimethamine content and circulated to all participating laboratories. The results obtained (Table I) were considered satisfactory and the Sub-Committee recommends that the method be used for the determination of pyrimethamine in medicated animal feeds.

TABLE I

DETERMINATION OF PYRIMETHAMINE IN A MEDICATED POULTRY FEED CONTAINING 4.9 mg kg^{-1}

Laboratory*	Pyrimethamine found/mg kg^{-1}	Recovery, %
A	4.2,4.4	86,90
B	4.4	90
	4.5†,4.6†	92,94
C	7.9	161
D	3.9,3.9	80,80
E	5.8†,5.6†	118,114
F	4.8,4.5	98,92
	4.5†,4.3†	92,88
G	4.8,4.2	98,96
H	5.1,5.1	104,104
I	4.4, 4.1	90,84
J	5.0, 5.6	102,114
Range (excluding results from laboratory C)	3.9 – 5.8	
Mean	4.6	
Standard deviation	0.5	

* Laboratories A–F were members of the EEC Group of Experts and Laboratories G–J others in the UK.

† These results were obtained on a second batch of medicated feed.

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SHORT PAPERS

Determination of Tungsten in Its Ores and Concentrates by Atomic-absorption Spectrometry

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Keywords: Tungsten determination; ores; atomic-absorption spectrometry

The Tariff Schedules of the United States[1] provide that tungsten ore be assessed at a rate dependent on the tungsten content. Samples of tungsten ore and concentrate received by this laboratory have been analysed by a gravimetric procedure based on precipitation of tungstic acid with cinchonine hydrochloride in acidic solution and subsequent ignition to tungsten(VI) oxide.[2] This is a time-consuming procedure, requiring 16 h of operating time for four samples; atomic-absorption spectrometry was investigated as a possible more economical alternative.

Atomic-absorption spectrometric methods for tungsten have been developed primarily for the analysis of alloys and silicate ores[3,4] containing only a few per cent. of tungsten or less. Methods for high concentrations of tungsten in ores were developed by Quin and Brooks[5] and Thomas *et al.*[6] and were also discussed by Scobbie.[7] Before examining one of these methods, an attempt was made to incorporate atomic-absorption spectrometry into the previously used gravimetric procedure. This yielded values that were often significantly different from the gravimetric procedure values. When the procedure of Quin and Brooks,[5] which utilises a mixture of nitric and hydrofluoric acids to dissolve the ore samples, was tried, 2–3 h were required to dissolve the sample and 2 h to evaporate the solution to dryness. This was considered to be impractically long and the fusion method of Thomas *et al.*[6] was chosen for sample preparation.

Experimental

Apparatus

Atomic-absorption spectrometer. Perkin-Elmer, Model 460, with 50-mm slot, dinitrogen oxide - acetylene burner and Varian Techtron hollow-cathode lamp, HCN - W. Operating conditions: dinitrogen oxide flow-rate, 10 l min^{-1}; acetylene flow-rate, 7 l min^{-1}; hollow-cathode lamp, 20 mA; wavelength, 400.8 nm; slit width, 0.7 nm.

Furnace. Thermolyne 2000, Thermolyne/Sybron Corporation.

Reagents

Tungsten standard solutions.

Primary stock solution, 10 mg ml^{-1}. Dissolve 17.95 g of sodium tungstate ($Na_2WO_4.2H_2O$) (Fisher Scientific ACS grade) in 1 l of water containing 100 ml of 10% sodium hydroxide solution.

Secondary stock solution, 1 mg ml^{-1}. Pipette 50 ml of primary stock solution into a 500-ml calibrated flask and dilute to volume with water.

Working solutions. Pipette 20, 40, 60 and 80 ml of secondary stock solution into separate 100-ml calibrated flasks, add 20 ml of 10% sodium sulphate solution and dilute to volume with water to give solutions of 200, 400, 600 and 800 μg ml^{-1}, respectively.

Procedure

Grind the samples to a mesh of 200 in an agate mortar. Dry for 1 h at 105 °C and allow to cool in a desiccator. Weigh 0.5 g–0.1 mg into a 50-ml platinum crucible. Add 3 g of sodium carbonate and mix thoroughly. Cover the mixture with a layer of sodium carbonate and fuse

for 5 min in an electric muffle furnace at 880 °C. Remove the crucible and let it cool to room temperature. Place the crucible in a 250-ml beaker and add enough water to cover it. Heat the beaker on a steam-bath or hot-plate to break the melt into a powder. Cool, filter through fast filter-paper into a 500-ml calibrated flask and wash the precipitate with water. Add 100 ml of 10% sodium sulphate solution and dilute to volume with water.

Measure the absorbance of this solution and of standard solutions that are 2% in sodium sulphate and contain tungsten in the range of concentrations present in the sample solution. The instrument is operated in the absorbance - hold mode using the following sequence: blank, low-concentration standard, blank, sample, blank, high-concentration standard. The absorbance is set to zero while aspirating the blank.

Two samples were analysed in duplicate at one time. For each sample, four to six separately weighed portions were analysed and each solution was measured ten times to collect enough data for statistical analysis.

Development of the Method

Method development and evaluation were performed on eight commercial samples of tungsten ores and concentrate previously analysed by the laboratory's gravimetric procedure[2] and one reference sample of tungsten(VI) oxide. Each sample was assayed by the gravimetric procedure at least twice in addition to the previous determination to produce more data for statistical analysis.

The first investigation was on whether it was possible to measure tungsten by atomic-absorption spectrometry after decomposing the ore in acidic solution as in the gravimetric procedure. Accordingly, after treating the sample with hydrochloric acid and then with hydrochloric plus nitric acids, the sample was evaporated to dryness, to expel the acids. The tungsten was dissolved by heating with potassium hydroxide solution, and the resulting tungsten-containing solution, after filtration and dilution, was used for atomic-absorption spectrometric measurements. The samples were run with[8] and without sodium sulphate. Table I, columns B, C and G, show the results of these analyses and the average gravimetric values. Acceptable results were obtained for only half of the samples by this procedure. It was believed that the tungsten did not completely dissolve in the potassium hydroxide solution. Fusion was then chosen to solubilise the tungsten utilising the method of Thomas *et al.*[6] The sample was fused with potassium hydroxide and after dispersion with water the melt was dissolved in orthophosphoric acid and hydrogen peroxide was added. It was observed during the fusion that there was considerable spattering that was difficult to control. Table I, column D, shows results from these determinations. It is believed that the low results obtained in half of the samples analysed are due, at least in part, to loss of sample through spattering.

TABLE I

TUNGSTEN CONTENT OF SAMPLES ANALYSED BY ATOMIC-ABSORPTION SPECTROMETRIC AND GRAVIMETRIC PROCEDURES

	Average W content by AAS, %						
	Acid dissolution			Na_2CO_3 fusion			
Sample (A)	Na_2SO_4 added (B)	Direct (C)	KOH fusion (D)	Direct (E)	Ca removed (F)	Average W content by gravimetric analysis, % (G)	Ca content by AAS, % (H)
1	46.2	42.3	53.0	49.9	53.1	53.5	3.3
2	27.7	22.3	41.3	47.7	52.3	52.6	11.4
3	38.9	41.3		39.9	40.7	41.5	0.6
4	59.3	60.2		58.3	58.3	59.3	0.3
5	49.3	55.8	55.2	53.9	55.1	55.7	0.7
6	52.3	54.7	53.8	52.9	53.3	54.1	0.5
7	56.0	56.0	54.9	58.5	58.8	59.5	0.5
8	50.4	54.6	53.3	56.2	55.6	56.8	0.7

The fusion procedure was altered by substituting sodium carbonate for potassium hydroxide and a platinum crucible for one made of zirconium. After dispersion with hot water and cooling to room temperature, the samples were treated with orthophosphoric acid and hydrogen peroxide. Sodium sulphate solution was added to sample and standard solutions. Atomic-absorption spectrometric measurements were made on these solutions and the results are given in Table I, column E. They are in close agreement with the gravimetric values except for samples 1 and 2.

It was thought that the accuracy of the data might be adversely affected by the presence of calcium in these samples, which could depress tungsten absorbance values. Therefore, calcium was determined in the ores and concentrates by atomic-absorption spectrometry[9] and the values are listed in Table I, column H. It can be seen that only samples 1 and 2 have significant calcium levels. As fusion of the sample with sodium carbonate presumably yields calcium carbonate, which is insoluble in a basic medium, the samples were not acidified with orthophosphoric acid after dispersion of the melt with hot water. Instead, they were transferred into calibrated flasks, sodium sulphate solution was added, the solutions were diluted and the absorbances measured by atomic-absorption spectrometry. The results are given in Table I, column F. They are slightly but consistently lower than the gravimetric data.

Other metallic elements that may be present in some of these samples at high concentration are iron and manganese. Samples not containing these elements at significant concentrations were made about 20% in iron or manganese by addition of appropriate salts. No significant effect on the tungsten results was observed. It should be noted that in the basic medium used, these elements, and many other potential interferents, are removed from the solution prior to aspiration. Thus, in the solution from the sample to which iron was added, no iron was detected when tested by atomic-absorption spectrometry. The added sodium sulphate also serves to eliminate interferences.

Precision and Accuracy

The averages of atomic-absorption spectrometric and gravimetric data were compared by Student's *t*-test, which showed that in most instances there is no significant difference between the data at the 95% confidence level. The calibration graph is linear up to 1000 μg ml^{-1} of tungsten and has an intercept of 0.0011 as determined by least-squares analysis. The standard error for the determination was found to be 0.001% of tungsten.

The accuracy of the atomic-absorption procedure was checked with a sample of tungsten(VI) oxide of purity greater than 99.99%. Measurements performed with ten separately weighed samples gave average results of 79.6% tungsten, compared with the 79.3% theoretical result. The relative accuracy was 0.38% and the relative standard deviation for these measurements was 1.25%. In Table II data are presented for the eight com-

TABLE II

SUMMARY OF ATOMIC-ABSORPTION SPECTROMETRIC DATA FOR TUNGSTEN CONTENT OF SAMPLES FUSED WITH SODIUM CARBONATE

		Samples analysed							
		Direct				Ca removed			
		Tungsten content, %		Relative standard	Number of	Tungsten content, %		Relative standard	Number of
Commodity	Sample	Average	Range	deviation, %	samples	Average	Range	deviation, %	samples
Tungsten concentrate	1	49.9	49.3–51.3	1.45	6	53.1	52.3–54.1	1.56	4
Scheelite	2	47.7	46.9–49.3	1.27	7	52.3	51.3–53.1	1.11	6
Wolframite	3	39.9	39.1–40.7	1.64	4	40.7	40.3–41.1	1.07	4
Tungsten concentrate	4	58.3	57.8–58.6	0.59	4	58.3	57.9–58.6	0.65	3
Wolframite	5	53.9	53.4–54.4	0.94	3	55.1	54.0–55.6	1.31	4
Tungsten ore	6	52.9	52.2–53.2	1.09	3	53.3	52.7–53.9	1.20	4
Tungsten ore	7	58.5	57.2–59.2	1.54	4	58.8	58.3–59.5	0.76	6
Tungsten ore	8	56.2	55.1–58.0	2.36	5	55.6	55.1–56.2	0.84	4

mercial samples analysed by the two procedures based on fusion with sodium carbonate. The number of separately weighed samples analysed, range of results and their average and percentage relative standard deviations are given for each of the eight samples. For the procedure that led to a removal of calcium before the atomic-absorption spectrometric measurements, the average relative standard deviation for the eight samples was 1.06%. The quality of these data compare favourably with those found in the literature.[5]

Four samples require about 3.5 h of actual operator time for atomic-absorption spectrometry compared with 16 h for the gravimetric analysis. It is concluded that this atomic-absorption spectrometric procedure is generally a satisfactory substitute for the gravimetric determination of tungsten in ores and concentrates.

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Differential-pulse Polarographic Determination of Degradation Products of Cephalosporins: Comparison of the Degradation of Cephaloglycin in Neutral Solution with that of Cephalexin

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Keywords: *Differential-pulse polarography*; *cephaloglycin*; *degradation*

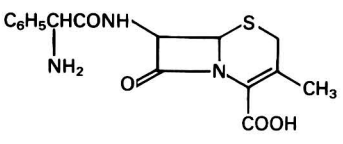
Differential-pulse polarography has been shown previously to be a useful technique for studying the degradation of cephalosporins in aqueous solution, and for determining cephalosporins and their degradation products.[1–4] Cephalexin (**I**) degrades in neutral solution by intramolecular aminolysis to give the diketopiperazine derivative (**II**), which gives a polarographic peak at −0.90 V (pH 7.4). Two further major peaks appear at −0.96 and −1.26 V owing to other degradation products.

The peak at −0.96 V appears late in the degradation and is due to a pyrazine compound: there is doubt as to whether this compound is 2-hydroxy-3-phenylpyrazine (**III**) or 2-hydroxy-3-phenyl-6-methylpyrazine (**IV**). The formation of this pyrazine derivative is the basis of a fluorimetric method for determining cephalexin and other α-aminobenzylcephalosporins and -penicillins.[5] When formaldehyde is added as a catalyst in this reaction it now seems clear that 2-hydroxy-3-phenyl-6-methylpyrazine is formed and it is probable that the formaldehyde is incorporated into the molecule as the 3-methyl group.[5,6] Previously, we have found that the formation of the pyrazine compound under solution conditions that give good yields is not

sufficiently reproducible for the reaction to be made the basis of a precise differential-pulse polarographic method for the determination of cephalexin.[3]

The peak at −1.26 V disappears on the addition of disulphite and is probably due to an unidentified carbonyl compound. The compound is formed early in the degradation of cephalexin and its formation appears to be associated with the degradation of the diketopiperazine compound (**II**). Cephalexin is degraded reproducibly and in high yield to this carbonyl compound by heating in pH 7.4 phosphate buffer solution at 100 °C for 1 h, and this procedure has been made the basis of a differential-pulse polarographic method for the determination of cephalexin.[3]

This paper describes a polarographic study of the degradation of cephaloglycin (**V**) in neutral and nearly neutral buffer solutions as a comparison with the degradation of cephalexin.



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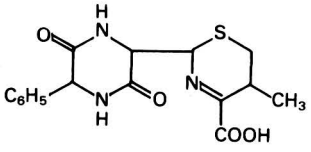

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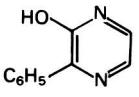
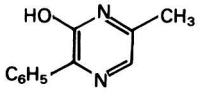


III



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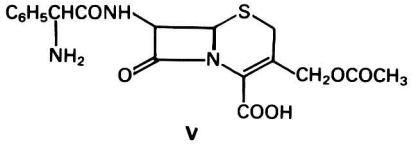
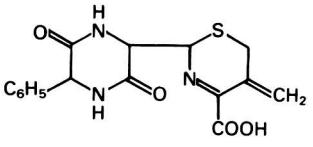


V



VI

Experimental

Differential-pulse polarography was carried out with a PAR 174A polarographic analyser (Princeton Applied Research) in the three-electrode mode using a dropping-mercury electrode, a platinum counter electrode and a saturated calomel reference electrode. A forced drop time of 1 s and a pulse amplitude of 50 mV were used. Polarography was carried out at 25 °C in a thermostatically controlled cell at pH 7.4 irrespective of the degradation pH, unless otherwise indicated.

Degradation studies were carried out with and without the removal of the hydrogen sulphide that formed during degradation. In the former instance, degradations were carried out in a gas wash-bottle whilst a slow stream of nitrogen gas, pre-saturated with water, was passed through the solution.

Samples of cephaloglycin and cephalexin were kindly provided by Lilly Research Centre Ltd. and Glaxo Operations (UK) Ltd., respectively.

Results

In pH 2 Britton - Robinson buffer solution (0.04 M in boric, orthophosphoric and acetic acids) cephaloglycin has a polarographic wave at −0.90 V, corresponding to the reductive elimination of the C-3 acetyl group.[7] The degradation of cephaloglycin at 37 °C in pH 7.4 buffer solution was followed by adjusting aliquots of the solution to pH 2 and measuring the height of this wave. At 37 °C the cephaloglycin in a 100 p.p.m. solution had almost disappeared after 10 h. During this time a peak appeared at −0.70 V. This peak reached a maximum height after 10 h and then became smaller as the compound responsible for it itself degraded (Figs. 1 and 2). Bundgaard[8] studied the degradation of cephalexin and cephaloglycin by ultraviolet spectrophotometry and other techniques and showed that the first degradation products are the diketopiperazine derivatives, compound **II** and compound **VI** for cephalexin and cephaloglycin, respectively. By recording the polarograms of partly degraded cephalexin and cephaloglycin solutions after adjusting the pH to various values, the dependence on pH of the half-wave potentials of the polarographic waves of the diketopiperazine (DKP) derivatives was determined. The half-wave potentials were found to vary rectilinearly with pH in the range 2–9.5 and to follow the equations $E_{\frac{1}{2}} = -0.15 - 0.103\text{pH}$ (DKP from cephalexin) and $E_{\frac{1}{2}} = -0.25 - 0.063\text{pH}$ (DKP from cephaloglycin).

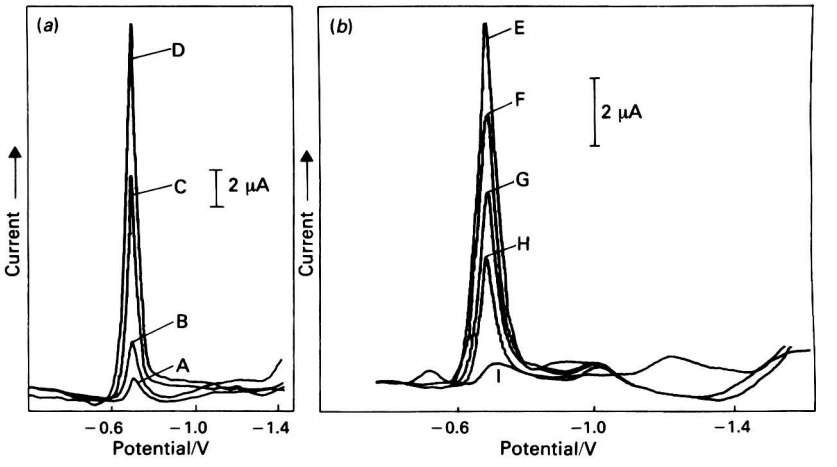


Fig. 1. Differential-pulse polarograms obtained for the degradation of a 100 μg ml^{-1} solution of cephaloglycin in pH 7.4 Britton - Robinson buffer at 37 °C in a stream of nitrogen. Degradation time: A, 20 min; B, 40 min; C, 4 h; D, 9 h; E, 25 h; F, 51 h; G, 66 h; H, 80 h; and I, 163 h.

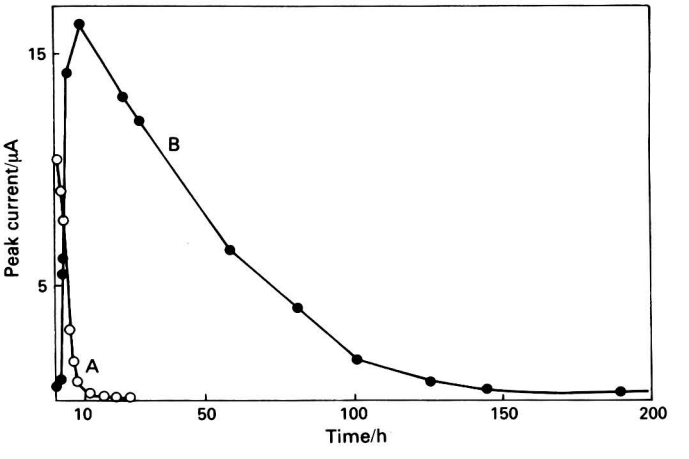


Fig. 2. Degradation of a 100 μg ml^{-1} solution of cephaloglycin in pH 7.4 Britton - Robinson buffer at 37 °C. A, Cephaloglycin peak at −0.90 V (polarographed after adjustment to pH 2); and B, diketopiperazine (**VI**) peak at −0.70 V (pH 7.4).

Other small waves were recorded in the polarograms of degraded cephaloglycin but no wave corresponding to that (at −1.26 V) of the carbonyl compound formed in the degradation of cephalexin was observed. This was checked at various stages of the degradation by the addition of disulphite: none of the waves, large or small, was affected markedly by its addition. This was also true when the degradation was carried out at 100 °C in conditions under which large yields of the carbonyl compound had been obtained for cephalexin.[2,3] Under these latter conditions the peak at −0.96 V owing to the pyrazine compound (**III** and/or **IV**) appeared (see Fig. 3), as had occurred with cephalexin.

As with cephalexin, degradation of cephaloglycin at pH 8.5 and 100 °C gave a greatly increased yield of the pyrazine derivative (see Fig. 4). Both compounds yielded a smaller wave at −1.07 V.

The pH dependence of the pyrazine peak obtained by the polarography of degraded cepha-

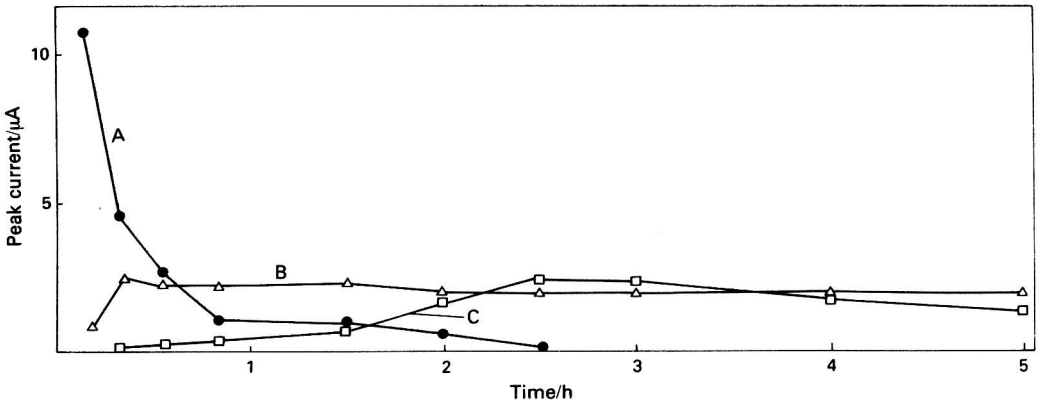


Fig. 3. Degradation of a 100 μg ml^{-1} solution of cephaloglycin in pH 7.4 Britton - Robinson buffer at 100 °C. A, Diketopiperazine (**VI**) peak at −0.70 V; B, pyrazine peak at −0.96 V; and C, hydrogen sulphide peak at −0.56 V.

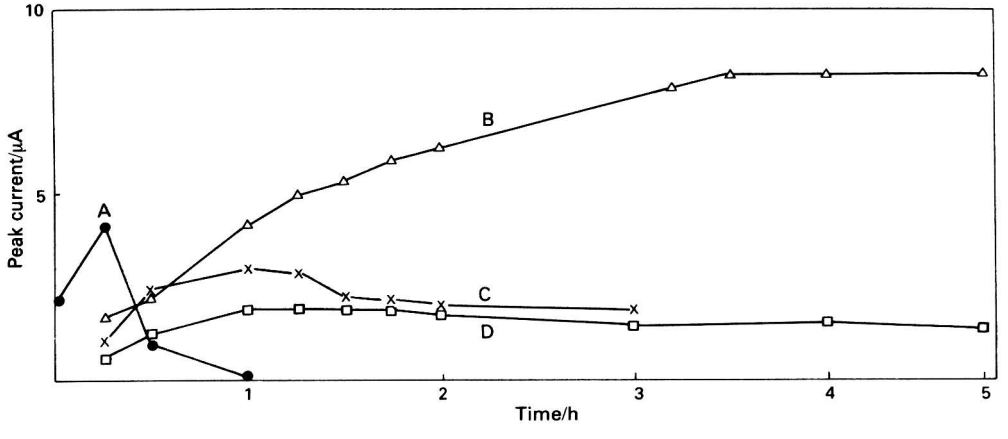


Fig. 4. Degradation of a 100 μg ml^{-1} solution of cephaloglycin in pH 8.5 phosphate buffer at 100 °C. Solution aliquots polarographed after adjustment to pH 7.4. A, Diketopiperazine (**VI**) peak at −0.70 V; B, pyrazine peak at −0.96 V; C, peak at −1.07 V; and D, hydrogen sulphide peak at −0.56 V.

lexin and cephaloglycin solutions between pH 2 and 10 was found to follow the equations $E_{\frac{1}{2}} = -0.50 - 0.065\text{pH}$ and $E_{\frac{1}{2}} = -0.48 - 0.068\text{pH}$, respectively, which are identical within experimental error. The peak of the carbonyl compound formed from cephalexin was found to be independent of pH ($E_{\frac{1}{2}} = -1.26$ V).

Discussion

Cephaloglycin can be determined polarographically by means of its polarographic wave at −0.90 V at pH 2. Cephalexin, which does not give a polarographic wave of its own, can be determined as its carbonyl degradation product at −1.26 V (pH 7.4).[3] If cephaloglycin forms an analogous carbonyl degradation compound it is not reduced within the available potential range. It had been intended to develop an alternative, more selective procedure for the polarographic determination of cephaloglycin based on the formation of this compound. The diketopiperazine primary degradation product of cephaloglycin is reduced at a potential that

differs greatly from that of cephalexin, and polarography of partially degraded solutions could be used to distinguish between the two compounds.

Both cephalosporins give hydrogen sulphide on degradation at pH 7.4. The amount produced from cephalexin was determined previously by blowing off the hydrogen sulphide into cadmium nitrate solution and determining it indirectly by the decrease in the height of the cadmium wave.[2] No attempt has been made to determine the amount produced at pH 7.4 from cephaloglycin by this means, but a spectrophotometric method is being developed for the determination of cephaloglycin and other cephalosporins based on the hydrogen sulphide formed during alkaline hydrolysis. This will be reported separately. Further, from alkaline degradation studies currently being undertaken, the peak obtained at −1.07 V (pH 7.4) during the degradation of cephaloglycin and cephalexin is now believed to arise from the degradation of their respective diketopiperazine derivatives. This will be reported later.

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Indirect Amplification Method for Determining Peroxydisulphate by Alternating-current Polarography

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Keywords: *Peroxydisulphate determination*; *amplification*; *polarography*

Polarographic techniques[1,2] have been reported for the determination of peroxydisulphate, which is a very strong oxidising agent. Using alternating-current (a.c.) polarography,[2] peroxydisulphate concentrations of about 10^{-3} M have been measured.

According to Müller and Von Ferber,[3] peroxydisulphate quantitatively oxidises iodide to iodate in an alkaline medium:

$$3S_2O_8^{2-} + I^- + 6OH^- \rightarrow 6SO_4^{2-} + IO_3^- + 3H_2O$$

If the solution is then acidified with dilute sulphuric acid, the iodate reacts with iodide in the usual manner to liberate iodine.

The increasing popularity of amplification reactions is not unexpected as they increase the sensitivity and improve the accuracy of the determination. This paper describes a method based on the reaction between peroxydisulphate and an excess of iodide in an alkaline medium; the iodate that is formed is acidified and the liberated iodine is extracted into chloroform, followed by back-extraction using sodium hydrogen sulphite solution[4] to reduce iodine to iodide; the iodide is then oxidised by bromine water to yield iodate. The iodate that is formed

undergoes reduction at the dropping-mercury electrode producing a single wave that corresponds to direct reduction to iodide as shown by the following equation[5]:

$$IO_3^- + 6H^+ + 6e^- \rightarrow I^- + 3H_2O$$

Thus, iodide can be determined after conversion into another species (iodate), which yields a wave six times greater than that of iodide with a one-electron transfer, *i.e.*, iodide can be determined after a 36-fold amplification.

Another amplification method involves the use of periodate.[6] Periodate oxidises iodide according to the equation

$$I^- + 3IO_4^- \rightarrow 4IO_3^-$$

Subsequent polarographic reduction of the iodate formed requires 144 electrons and, therefore, gives a 144-fold amplification for the determination of iodide.

Experimental

Apparatus

Any suitable polarograph, *e.g.*, a Metrohm E506 Polarecord, with a three-electrode electrolytic cell. The working electrode is a dropping-mercury electrode fitted with a tapping device for controlling drop times to 0.4–0.6 s; the reference electrode is silver - silver chloride, potassium chloride and the auxiliary electrode is a platinum wire.

Reagents

All reagents used were of analytical-reagent grade.

Standard peroxydisulphate solution, 0.5 mg ml.$^{-1}$ Dissolve 0.351 75 g of potassium peroxydisulphate in distilled water and make up to 500 ml. Prepare less concentrated solutions by dilution.

Potassium iodate, standard solutions. Dissolve 170 mg of potassium iodate in 1 l of water to give a 0.1 mg ml^{-1} iodide solution. Also dissolve 1000 mg of potassium iodate in 1 l of water to give a 0.6 mg ml^{-1} iodide solution. These solutions were used for constructing calibration graphs. The straight-line calibration graphs pass through the origin and therefore satisfy the Ilkovič equation. The calibration graphs should be checked with each new set of potassium iodate standard solutions.

Potassium periodate solution. This was freshly prepared for each determination by dissolving 350 mg of potassium periodate in 20 ml of hot water, adding 0.6 ml of saturated disodium tetraborate solution and making up to 100 ml with water. It is kept in an amber-glass bottle.

Bromine water. A saturated solution.

Formic acid. An 80% solution.

Ammonium chloride solution, 10% .

Zinc acetate solution, 10%.

Sodium hydrogen sulphite solution, 0.5%.

Gelatin solution, 1%. Prepared every week.

Sodium hydroxide solution, 2 M.

Sulphuric acid, 4 N.

Oxygen-free nitrogen. Obtained by passing the stream of gas through a purification system consisting of three bubblers containing pyrogallol followed by one bubbler containing 1 N hydrochloric acid and one empty bubbler.

Procedure

Into a 50-ml glass-stoppered Erlenmeyer flask, introduce 5 ml of 2 M sodium hydroxide solution and 0.2–1.0 g of potassium iodide followed by 10 ml of water. After the iodide has dissolved, add a suitable volume of the sample solution (containing 10–2000 μg of peroxydisulphate) and boil the reaction mixture gently for 1 min, then cool. Transfer the solution quantitatively into a 100-ml separating funnel and acidify with 3 ml of 4 N sulphuric acid. Extract the liberated iodine with three 15-ml portions of chloroform Collect the extracts in another funnel. Add 10 ml of water and shake to remove trace amounts of iodide. Discard the aqueous layer and shake the chloroform - iodine solution with 0.5 ml of sodium hydrogen sulphite solution, and 10 ml of water, to reduce iodine to iodide. Transfer the aqueous layer,

containing iodide, into a 50-ml conical flask, then determine it using one of the following methods.

36-Fold amplification method.

To the iodide solution, add 2 ml of bromine water and stir for 5 min. Introduce 0.5 ml of formic acid solution and stir for 5 min. Transfer quantitatively into a 50-ml calibrated flask; add 6 ml of 2 M sodium hydroxide solution, 1 ml of gelatin solution and dilute with water up to the mark. After bubbling nitrogen through the solutions, polarograph an aliquot of the solution starting from −1 V, *versus* the silver - silver chloride electrode.

Following the same steps, record the iodate wave of a blank solution using the same amount of iodide but with no peroxydisulphate. Compare the height of each of the blank and sample waves with the calibrated graphs constructed for iodate to obtain the amount of iodide reacted with peroxydisulphate.

144-Fold amplification method

Oxidise the iodide solution by adding 8 ml of the periodate solution; stopper and allow to stand for 5 min at room temperature (25 °C) and then immerse the flask in a boiling water-bath for 30 min. Cool to room temperature, add 2 ml of 10% ammonium chloride solution followed by 2 ml of 25% ammonia solution. Heat just to boiling and precipitate the excess of periodate by adding 5 ml of 10% zinc acetate solution. Leave the mixture undisturbed for 30 min at room temperature. Filter off the zinc periodate using a Whatman No. 42 filter-paper and wash the precipitate with two 5-ml portions of distilled water. Collect the filtrate containing the iodate and washings in a 50-ml calibrated flask. Add 1 ml of gelatin solution followed by 1.5 ml of 4 N sulphuric acid and dilute to the mark with water. Shake and transfer an aliquot of the solution into the polarographic vessel. Bubble nitrogen through the solution and record the iodate wave starting at −1 V *versus* the silver - silver chloride electrode. Under the same conditions, record the iodate wave of a blank solution using the same amount of iodide but no peroxydisulphate. Compare the height of each of the blank and sample waves with the iodate calibration graphs to obtain the amount of iodide that had reacted with peroxydisulphate.

Calculate the mass of peroxydisulphate using the equation

$$\text{Mass of peroxydisulphate (mg)} = \frac{3 \times \text{relative molecular mass of } S_2O_8^{2-} \times M}{\text{relative atomic mass of } I^-}$$
$$= 4.535 \times M$$

where M = mass (in milligrams) of iodide that had reacted with peroxydisulphate as calculated from the corresponding calibration graph.

For the 36- and 144-fold amplification methods, divide the mass of peroxydisulphate obtained by 6 and 24, respectively.

Calibration graph

The preparation of calibration graphs from a primary standard solution of potassium iodate was carried out as follows. To a series of 50-ml calibrated flasks, a suitable volume of standard potassium iodate solution, containing 0.5–150 p.p.m. of iodate, was introduced. A 2-ml aliquot of saturated bromine water and 0.5 ml of 80% formic acid were added, followed by 6 ml of 2 M sodium hydroxide solution and 1 ml of 1% gelatin solution. The solutions were then diluted to 50 ml with water, and the polarographic iodate wave for each solution was measured starting at −1 V *versus* the silver - silver chloride electrode. A reagent blank was prepared similarly. An increase in the wave height was observed by increasing the iodate concentration. The current - concentration graphs were linear over the concentration range measured, passing through the origin and therefore satisfying the Ilkovič equation.

Results and Discussion

This method depends on using an excess of iodide to react quantitatively with peroxydisulphate in an alkaline medium. This quantitative reaction has been proved experimentally by heating the reaction mixture; the reaction of peroxydisulphate and iodide is accelerated by

heating and if a large excess of iodide is present.[7] It was found that 0.2–1.0 g of iodide gave good results if the reaction mixture is heated to gentle boiling for 1 min. Solutions of pH less than 7 gave low results owing to the liberation of iodine and its loss upon heating the reaction mixture. However, highly alkaline solutions gave rise to high results owing to the formation of hypoiodite. Satisfactory results were obtained by adding 5 ml of 2 M sodium hydroxide solution.

Removal of the excess of periodate in the 144-fold amplification method has been tried by adding molybdate solution[8] at pH 3, but the polarographic wave of molybdenum interfered with that of iodate. Zinc acetate removed the excess of periodate, as insoluble zinc periodate, from an alkaline medium.[9] Recording the iodate wave without filtering the solution was not feasible as zinc produces a wave in the alkaline medium used, which interferes with that of iodate. After the separation of zinc periodate, acidification with sulphuric acid eliminated the interference owing to zinc.

Although a.c. polarography is useful for determining peroxydisulphate at concentrations of about 10^{-3} M, the proposed amplification procedure enables analysis of a peroxydisulphate solution as dilute as 2.6×10^{-6} M with reasonable accuracy. The relative error did not exceed 2%. For 10, 100 and 2000 μg of peroxydisulphate (three replicates), the 36-fold method gave relative standard deviations of 1.65, 0.90 and 0.58%, respectively (Table I); for 10, 100 and 2000 μg of peroxydisulphate (three replicates), the 144-fold method gave relative standard deviations of 2.10, 0.84 and 1.50%, respectively (Table II). The recoveries were 1–2% low for lower concentrations, this may be owing to a loss of iodine during the extraction process. However, the 36-fold amplication method seems to be simpler and less time consuming than the 144-fold amplification method; one determination requires 30 min. Metal ions that form water-insoluble iodides [*e.g.*, silver(I), lead(II) and copper(I)] are expected to interfere; also among the interfering substances are organic materials, ammonium salts and hydrogen peroxide. Organic substances that neither react with iodine nor oxidise iodide do not interfere in concentrations of up to 1 M. Similarly, substances that yield polarographic waves from sodium hydroxide [*e.g.*, zinc(II)] or the sulphuric acid supporting electrolyte [*e.g.*, molybdenum(VI), copper(II) and bismuth(III)] that have half-wave potentials close to that of iodate, also interfere.

TABLE I

MICRO-DETERMINATION OF PEROXYDISULPHATE BY THE 36-FOLD AMPLIFICATION METHOD

Amount of $S_2O_8^{2-}$/μg			
Taken	Found *	Relative error, %	Relative standard deviation, %
10	9.8	−2.00	1.65
30	29.6	−1.33	
50	50.2	+0.40	
100	100.4	+0.40	0.90
500	500.3	+0.06	
1000	995.0	−0.50	
2000	1970.0	−1.50	0.58

* Mean of three determinations.

TABLE II

MICRO-DETERMINATION OF PEROXYDISULPHATE BY THE 144-FOLD AMPLIFICATION METHOD

Amount of $S_2O_8^{2-}$/μg			
Taken	Found*	Relative error, %	Relative standard deviation, %
10	9.9	−1.00	2.10
30	29.8	−0.67	
50	50.1	+0.20	
100	100.3	+0.30	0.84
500	500.4	+0.08	
1000	999.0	−0.10	
2000	1985.0	−0 75	1.50

* Mean of three determinations.

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Titrimetric Micro-determination of Peroxydisulphate by Amplification Reactions

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Keywords: *Peroxydisulphate determination*; *amplification*; *iodimetry*

Classical[1,2] and instrumental[3,4] methods are available for the determination of peroxydisulphate (persulphate), which is a very strong oxidant. In neutral solution, peroxydisulphate reacts with iodide to liberate an equivalent amount of iodine:

$$S_2O_8^{2-} + 2I^- \rightarrow 2SO_4^{2-} + I_2$$

The time required for the complete reaction of peroxydisulphate depends on the iodide concentration and on the ionic strength.[5] According to Müller and von Ferber,[6] peroxydisulphate oxidises iodide quantitatively to iodate in alkaline medium:

$$3S_2O_8^{2-} + I^- + 6OH^- \rightarrow 6SO_4^{2-} + IO_3^- + 3H_2O$$

If the solution is then acidified with dilute sulphuric acid, the iodate reacts with iodide in the usual way.

In order to develop a cheap, reliable and sensitive method (necessary because low concentrations of peroxydisulphate can be used as a flour improver) we have investigated the combination of a titrimetric method with an amplification procedure for the determination of peroxydisulphate, based on the reaction between peroxydisulphate and an excess of iodide in alkaline medium; the iodate formed is acidified and the liberated iodine is extracted into chloroform and then reduced to iodide. The resulting iodide is determined by the Leipert amplification procedure giving a 6-fold amplification[7]:

$$I^- \xrightarrow{Br_2} IO_3^- \xrightarrow{5I^- + 6H^+} 3I_2$$

$$3I_2 \xrightarrow{NaHSO_3} 6I^- \xrightarrow{Br_2} 6IO_3^- \xrightarrow{KI + H_2SO_4} 18I_2$$

Alternatively, the iodide formed is oxidised by periodate[8]:

$$I^- + 3IO_4^- \rightarrow 4IO_3^-$$

The excess of periodate is masked with molybdate[9] and the iodate formed is reacted with iodide to give a 24-fold amplification:

$$6I^- \xrightarrow{\text{Periodate}} 24IO_3^- \xrightarrow{KI + H_2SO_4} 72I_2$$

The method is more sensitive than the classical methods,[1,2] being applicable to the determination of 0.02-mg amounts of peroxydisulphate, whereas the titrimetric method reported by Kolthoff and Carr[2] was suitable for 6–30-mg amounts. Some of the instrumental methods[3,4] are sensitive to temperature changes, depend on unstable colours and also require instruments that may not be available in every laboratory.

Experimental

Reagents

All chemicals used were of analytical- or microanalytical-reagent grade.

Standard peroxydisulphate solution, 1 mg ml^{-1}. Dissolve 0.7035 g of potassium peroxydisulphate in distilled water and dilute to 500 ml. Prepare less concentrated solutions by dilution.

Sodium thiosulphate solutions, 0.002, 0.004, 0.02 *and* 0.04 N. Standardise against potassium iodate solutions of similar normality.

Sulphuric acid, 4 N.

Sodium hydroxide solution, 2 M.

Sodium hydrogen sulphite solution, 1% *m/V*.

Potassium periodate solution, 0.015 M. Dissolve 1.75 g of the salt in 500 ml of water, add 3 ml of saturated borax solution and store in a dark glass bottle.[8] Prepare fresh as required.

Ammonium molybdate solution, 25% *m/V*. Freshly prepared.

Acetate buffer solution, pH 2.2. Prepare by mixing 8 ml of 0.2 M sodium acetate solution and 20 ml of glacial acetic acid.

Formic acid, 80% *m/V*.

Bromine water, saturated.

Apparatus

Grade B micro-burettes (2- and 5-ml capacities).
Grade A micro-pipettes (0.1-, 1- and 2-ml capacities).
Glass-stoppered Erlenmeyer flasks (50-ml capacity).

Procedure

In a 50-ml glass-stoppered Erlenmeyer flask, place 5 ml of 2 M sodium hydroxide solution and 0.2–1.0 g of potassium iodide followed by 5 ml of distilled water. After the iodide has dissolved, add a suitable volume of peroxydisulphate solution (containing 0.02–3.0 mg of peroxydisulphate), boil the solution gently for 1 min, then cool. Transfer the solution into a 100-ml separating funnel and acidify with 3 ml of 4 N sulphuric acid. Extract the liberated iodine with three 20-ml portions of chloroform or benzene. Collect the extract in another funnel and wash the combined extract with 5 ml of distilled water to remove any iodide carried over. Shake the iodine solution with 10 ml of distilled water containing 0.5 ml of 1% sodium hydrogen sulphite solution to reduce iodine to iodide. Transfer the aqueous layer, containing the iodide, into a 50-ml conical flask, then determine it using one of the following methods. Run a blank determination.

6-*Fold amplification*

Add 2 ml of saturated bromine water to the iodide solution. Stopper the flask and stir the solution for 5 min. Destroy the excess of bromine by adding 1 ml of 80% *m/V* formic acid solution and stirring for 5 min. Add 2.5 ml of 4 N sulphuric acid and about 0.5 g of potassium iodide, and titrate the liberated iodine with 0.02 N sodium thiosulphate solution in the usual way using starch as an indicator. For low concentrations (less than 0.5 mg of peroxydisulphate) use 0.002 N thiosulphate solution.

24-*Fold amplication*

Add 10 ml of periodate solution to the iodide solution. Stopper the flask and allow the reaction mixture to stand for 5 min at room temperature, then place the flask in a boiling

water-bath for 30 min for complete oxidation. Cool, add 10 ml of ammonium molybdate solution and 5 ml of pH 2.2 acetate buffer solution. Add about 0.5 g of potassium iodide; stopper the flask and set it aside for 5 min. Titrate the liberated iodine with 0.04 N thiosulphate solution. For low concentrations (less than 0.5 mg of peroxydisulphate) use 0.004 N thiosulphate solution.

The amount of peroxydisulphate is calculated using the equation

$$1 \text{ mg } I^- = 4.5418 \text{ mg } S_2O_8^{2-}$$

$$\text{Amount of } S_2O_8^{2-} \text{ (mg)} = \frac{4.5418(S - B)}{F}$$

where S = volume of thiosulphate consumed in the sample run, B = volume of thiosulphate consumed in the blank run (0.46 ml of 0.002 N and 0.38 ml of 0.004 N thiosulphate solutions used for the 6- and 24-fold amplification methods, respectively), and F = volume of thiosulphate equivalent to 1 mg of iodide originally present (14.2 ml of 0.02 N and 28.4 ml of 0.04 N thiosulphate solutions used for 6- and 24-fold amplification methods, respectively).

Results and Discussion

Under the experimental conditions used, the peroxydisulphate reacts with iodide in an alkaline medium. This has been proved experimentally by heating the reaction mixture and boiling for about 1 min. Solutions with pH values of less than 7 give low results owing to the liberation of iodine and its loss upon heating the reaction mixtures. However, a highly alkaline medium gave high results owing to the formation of hypoiodite. It was found that 5 ml of 2 M sodium hydroxide solution gave the optimum pH. The reaction of peroxydisulphate and iodide is accelerated by heating and by the presence of a large excess of iodide.[10] It was found that 0.2–1.0 g of iodide gave good results if the mixture is allowed to boil for 1 min.

The two amplification methods gave satisfactory results, the recoveries ranging from 98 to 100.1% for the 6-fold amplication method (0.05–3.0 mg of peroxydisulphate (Table I) and from 95 to 100% for the 24-fold amplification method (0.02–3.0 mg) (Table II). The recoveries were 2–5% low for the lower concentrations, possibly owing to loss of iodine during the extraction process. However, the 6-fold amplification method was simpler and less time consuming than the 24-fold amplification method, one determination taking about 30 min. The coefficients of variation for the 6-fold amplification method were 2.0, 0.9 and 0.5% for 0.05, 0.5 and 3.0 mg of peroxydisulphate (five replicates), respectively. For 24-fold amplification, the iodide was oxidised with periodate in borate buffer solution at pH 7 as recommended by Belcher *et al.*,[8] who described the determination of 10–200 μg of iodide. It was found that up to 8 mg of iodide can be oxidised quantitatively with 10 ml of 0.015 M periodate. The unused periodate is masked by molybdate[9] at pH 3–3.5; 10 ml of 25% *m*/*V* molybdate solution does not affect the final determination. The method is applicable to the determination of 0.02–3.0 mg of peroxydisulphate; larger amounts can be determined provided that the iodide, after oxidation to iodate, liberates an amount of iodine extractable

TABLE I

MICRO-DETERMINATION OF PEROXYDISULPHATE BY THE 6-FOLD AMPLIFICATION METHOD

$S_2O_8^{2-}$/mg		Recovery,	Coefficient of
Taken	Found*	%	variation, %
0.050	0.049	98.0	2.0
0.100	0.098	98.0	1.9
0.300	0.297	99.0	1.5
0.500	0.498	99.6	0.9
1.000	0.998	99.8	0.7
2.000	2.002	100.1	0.5
3.000	2.995	99.8	0.5

* Means of five determinations.

into three 20-ml portions of chloroform. The coefficients of variation for the 24-fold amplification method were 1.9, 0.8 and 0.3% for 0.02, 0.5 and 3 mg of peroxydisulphate (five replicates), respectively.

Reduction of iodine to iodide was achieved using 0.5 ml of 1% sodium hydrogen sulphite solution; larger amounts of the reductant reduce the iodine liberated in the final stage of the procedure.

TABLE II

MICRO-DETERMINATION OF PEROXYDISULPHATE BY THE 24-FOLD AMPLIFICATION METHOD

$S_2O_8^{2-}$/mg		Recovery,	Coefficient of
Taken	Found*	%	variation, %
0.020	0.019	95.0	1.9
0.050	0.049	98.0	1.7
0.100	0.099	99.0	1.2
0.500	0.499	99.8	0.8
1.000	1.000	100.0	0.6
2.000	2.000	100.0	0.4
3.000	2.996	99.9	0.3

* Means of five determinations.

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Indirect Spectrophotometric Determination of Iodide in Table Salts and Pharmaceutical Products

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Keywords: Iodide determination; 3-(2′-thiazolylazo)-2,6-diaminotoluene reagent; palladium complex; spectrophotometry

Several spectrophotometric methods have been recommended for the determination of iodide,[1,2] although the catalytic method developed by Sandell and Kolthoff[3,4] seems to be the most widely used.

This paper describes a procedure for the indirect spectrophotometric determination of iodide, based on the reaction of palladium with 3-(2′-thiazolylazo)-2,6-diaminotoluene (2,6-TADAT).[5] The determination involves the formation of a complex between palladium and iodide ions and the formation of a blue complex between unreacted palladium and 2,6-TADAT. The colour develops in 1 h and is stable for at least 24 h. A decrease in absorbance is observed with increasing iodide concentration.

The method has been applied to the determination of iodide in iodinated table salts and in pharmaceutical products.

Experimental

Apparatus

The apparatus used included a Beckman 25 recording spectrophotometer with silica cells of 1-cm path length, and a Radiometer PHM64 digital pH meter with glass and saturated calomel electrodes.

Reagents

Analytical-reagent grade chemicals were used throughout, without further purification.

3-(2′-*Thiazolylazo*)-2,6-*diaminotoluene*, 10^{-3} M *in* 1 M *perchloric acid.*

Palladium(II) perchlorate solution, 10^{-2} M *in* 1 M *perchloric acid.* This solution was standardised gravimetrically using dimethylglyoxime.

Sodium iodide solution, 10^{-2} M. Standardised titrimetrically using thiosulphate solution.

All of these solutions were diluted with distilled water as required.

Recommended Procedure for the Determination of Iodide

Into a 25-ml calibrated flask pipette 2 ml of 10^{-4} M palladium perchlorate solution, 2 ml of the 10^{-3} M solution of 2,6-TADAT in 1 M perchloric acid, a suitable volume of the iodide sample solution containing 12.7–63.5 μg of iodide and 6 ml of 60% perchloric acid and then dilute to volume with distilled water. After 1 h measure the absorbance of the solution at 590 nm against a reagent blank, in 1-cm path length cells.

Determination of Iodide in Iodinated Table Salts

Weigh accurately 15–30 g of the table salt, dissolve in water in a 100-ml calibrated flask and dilute to volume. Analyse suitable aliquots as described above.

Determination of Iodide in Pharmaceutical Products

Weigh accurately 0.2–2.0 g of the pharmaceutical product, mix well with 8–10 g of anhydrous sodium carbonate in a porcelain dish and ignite for 24 h at 420 °C. The cooled reaction mixture must be carefully decomposed and neutralised with 4 M perchloric acid, and diluted with distilled water in a 50- or 100-ml calibrated flask. Analyse suitable aliquots as described previously.

Results and Discussion

In solutions where the concentration of the ligand is 1.60×10^{-4} M, the concentration of palladium is 8.0×10^{-5} M and H_0 for perchloric acid is −1.0, the absorbance is inversely proportional to iodide concentration in the range 0.51–2.30 p.p.m., and regression analysis of the experimental results gives the following equations:

$$A = 0.66 - 0.192[I^-] \text{ (p.p.m.)}$$

$$A = 0.66 - 2.44 \times 10^4[I^-] \text{ (mol l}^{-1}\text{)}$$

The optimum concentration range, as evaluated by Ringbom's method, is 0.51–1.52 p.p.m. The method was applied to a series of ten samples in which the iodide content was 1.27 p.p.m. and the results show a mean value of 1.27 p.p.m. with a standard deviation of 7.4×10^{-3} p.p.m.

As shown in Table I, the method is highly selective, as only thiocyanate and oxidising ions, such as iodate, bromate and hexacyanoferrate(II), interfere seriously. Among the other halide ions, chloride and bromide do not interfere even if present in amounts greater than 25000 and 1000 times the actual concentration of iodide, respectively. The main limitation to the method is the formation of insoluble potassium perchlorate in such a highly concentrated perchloric acid medium.

The results obtained for three determinations of iodide in each of two samples of three iodinated table salts together with the result obtained for the standard salt issued by the Comité Européen d'Étude du Sel, are given in Table II where they are compared with the results obtained by application of the titrimetric method of Sadusk and Bail,[6] as recommended by Kolthoff *et al.*[7]

TABLE I

DETERMINATION OF IODIDE IN THE PRESENCE OF VARIOUS IONS

The amount of iodide taken in each determination was 1.27 p.p.m.

Ion	Molar ratio of ion to iodide	Iodide found,* p.p.m.	Ion	Molar ratio of ion to iodide	Iodide found,* p.p.m.
NO_3^-	200	1.27	Citrate	200	1.27
SO_4^{2-}	200	1.27	Tartrate	150	1.28
F^-	200	1.27	SCN^-	1	1.14
Cl^-	25000	1.27	IO_3^-	1	1.13
Br^-	1000	1.27	BrO_3^-	1	1.11
$H_2PO_4^-$	200	1.28	$Fe(CN)_6^{4-}$	1	1.14
CH_3COO^-	200	1.27	$Fe(CN)_6^{3-}$	1	1.13
$C_2O_4^{2-}$	200	1.29	Na^+	25000	1.27
MoO_4^{2-}	35	1.29	K^+	50	1.25
WO_4^{2-}	20	1.27	NH_4^+	100	1.25
VO_3^-	20	1.25	Ca(II)	75	1.27
AsO_4^{3-}	10	1.27	Mg(II)	100	1.27

* Mean of three determinations.

TABLE II

DETERMINATION OF IODIDE IN IODINATED TABLE SALTS

Sample	Iodide found, %	
	2,6-TADAT*	Volumetric analysis[6]†
Standard	3×10^{-4}	—
I	1.34×10^{-3}	$(1.35 \pm 0.11) \times 10^{-3}$
II	1.67×10^{-3}	$(1.79 \pm 0.18) \times 10^{-3}$
III	2.37×10^{-3}	$(2.39 \pm 0.09) \times 10^{-3}$

* Mean of two determinations.
† Results for ten determinations.

The method developed can also be applied to the determination of iodide in pharmaceutical products, in which it can be found as inorganic iodide as well as organic iodide, after decomposing the sample by igniting with anhydrous sodium carbonate under controlled conditions. Some results are shown in Table III, where they are compared with the results obtained by the catalytic method of Jwasaki *et al.*[8]

The ease of application of the indirect spectrophotometric determination of iodide is clearly an advantage compared with other spectrophotometric methods.

TABLE III

DETERMINATION OF IODIDE IN PHARMACEUTICAL PRODUCTS

Sample (approximate composition)	Form of iodide	Iodide found,* %	
		2,6-TADAT	Catalytic method[8]
Protein complex	Uncertain	0.027 1	0.026 2
Vitamin complex	KI	0.067 1	0.065 1
Mineral complex	MgI_2	0.001 7	0.001 8
Antibiotic	Organic	0.206	0.207
Eye drops	Organic	0.115	0.112
Syrup	Organic	0.833 mg ml^{-1}	0.832 mg ml^{-1}

* Mean of two determinations.

We thank Dr. Rafols (Unión Salinera Española), who supplied us with the sample of the standard salt of the Comité Européen d'Étude du Sel, and Cyanamid, Nezel, Berenguer-Beneyto, Juste, Promesa and Clariana-Picó, Pharmaceutical Laboratories, who kindly offered us samples of their products.

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Interference Due to Crystal Formation in the Spectrophotometric Determination of Iron(II) Using 2,4,6-Tri(2′-pyridyl)-1,3,5-triazine

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Keywords: *Iron(II) determination*; *spectrophotometry*; 2,4,6-*tri*(2′-*pyridyl*)-1,3,5-*triazine*

In the course of limnological investigations, crystals were observed to form during the spectrophotometric determination of iron(II) in aqueous solutions using 2,4,6-tri(2′-pyridyl)-1,3,5-triazine (TPTZ). The crystals were first noticed about 15 min after the reagents had been added and interfered in the absorptiometric determination of the iron(II) - TPTZ complex. The presence of crystals has not been reported before in limnological methods using TPTZ[1–3] and their appearance was therefore investigated.

Experimental

Reagents

All reagents were of AnalaR grade, except for TPTZ.

Acetate buffer, 2 M. Dissolve 272 g of sodium acetate ($CH_3COONa.3H_2O$) and 115 ml of glacial acetic acid in 1 l of distilled water.

Hydroxylammonium chloride. Dissolve 10 g in 100 ml of distilled water.

TPTZ solution, 5 mM. Dissolve 0.1651 g of TPTZ monohydrate (BDH Chemicals) in 100 ml of 0.1 M hydrochloric acid.

Iron(II) solution. Dissolve 0.7021 g of ammonium iron(II) sulphate [$FeSO_4(NH_4)_2SO_4.6H_2O$] in 1 l of distilled water acidified with 5 ml of concentrated sulphuric acid to give a stock solution containing 100 μg ml^{-1} of iron(II).

Procedure

Epilimnetic lake samples were filtered through pre-washed (250 ml of distilled water) Whatman GF/C filters (7 cm).

A 40.0-ml sample or standard was pipetted into a 50-ml calibrated flask and 5.0 ml of acetate buffer, 2.0 ml of reducing agent (where specified) and 2.0 ml of TPTZ were added sequentially and the volume was made up to the mark with distilled water. The pH of this solution was 4.5. After various time intervals at room temperature (24 °C) the absorbance was read at 595 nm in 4-cm cuvettes against distilled water.

Results and Discussion

The appearance of crystals with 2 ml of TPTZ is evident from Table I, in which the absorbance measured 5–10 min after the reagents had been added is compared with the absorbance after 60–70 min. The small, colourless crystals appeared about 15 min after the reagents had been added and increased in amount with time. The crystals were less evident in the distilled water solution and the water sample to which hydroxylammonium chloride had been added than in the solutions without the reducing agent. The crystals were also present if the hydroxylammonium chloride was replaced with ascorbic acid (100 g l^{-1}). Centrifugation resulted in clear solutions but crystals that had adhered to the sides of the centrifuge tube above the level of the solution were re-suspended on transfer of the solution into a cuvette. Reduction of the volume of TPTZ to 0.9 ml prevented the formation of crystals over a period of 60 min.

TABLE I

EFFECT OF DIFFERENT VOLUMES OF TPTZ ON THE ABSORBANCE OF THE IRON(II) - TPTZ COMPLEX IN VARIOUS SOLUTIONS

	Time after addition of reagents/min			
	2 ml of TPTZ		0.9 ml of TPTZ	
Solution	5–10	60–70	5–10	60–70
0 μg l^{-1} of Fe	0.006	0.203*	0.004	0.004
0 μg l^{-1} of Fe + RA†	0.018	0.028*	0.019	0.016
100 μg l^{-1} of Fe(II)	0.149	0.204*	0.150	0.151
100 μg l^{-1} of Fe(III) + RA	0.150	0.203*	0.149	0.149
Water sample	0.012	0.078*	0.010	0.014
Water sample + RA	0.031	0.042*	0.030	0.029

* Crystals present.
† RA = hydroxylammonium chloride added as reducing agent.

The molar absorptivity (ϵ) of the iron(II) - TPTZ complex was calculated as 22700 l mol^{-1} cm^{-1} using absorbance values determined 5 min after the reagents had been added. This value agrees well with literature values of 22300 (at 595 nm)[2] and 22600 l mol^{-1} cm^{-1} (at 593 nm).[4] The increase in the absorbance with time due to the formation of crystals was not observed if 2,2′-dipyridyl, FerroZine [monosodium 3-(2-pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine monohydrate] or bathophenanthroline disulphonate (disodium 4,7-diphenyl-1,10-phenanthroline disulphonate) was used as the iron(II) complexing agent.

It has been reported[5] that a break in the linear relationship between absorbance (285 nm) and TPTZ concentration occurs at 92 μM at pH 4.5; at this point a constant amount of dissolved TPTZ was indicated. The final iron(II) concentrations in the present study ranged from 0 to 1.4 μM and the final TPTZ concentration was 200 μM using 2 ml of TPTZ and 90 μM using 0.9 ml of TPTZ. This suggested that the crystals were uncombined TPTZ, insoluble at pH 4.5, as TPTZ chelates iron(II) in a molar ratio of 2 : 1.[4] In a study[6] of the determination of sulphur dioxide by reduction of iron(III) and the formation of iron(II) - TPTZ it was necessary to incorporate propylene carbonate to prevent precipitation of crystals that were assumed to be TPTZ. The authors did not specify at what concentrations of uncombined TPTZ the crystals appeared.

There were no crystals in the 5 mM stock TPTZ solution that was prepared in 0.1 M hydrochloric acid, indicating that the solubility of TPTZ is related to pH. Increasing the pH of this solution with alkali induced the formation of crystals that were very similar in appearance to those observed in the experimental flasks. These crystals could be redissolved by further acidification of the solution.

Methods involving the determination of iron have used final TPTZ concentrations of 100–200 μM[4] and 192 μM[1,3] (assuming a relative molecular mass for TPTZ of 312.3). Dougan and Wilson[2] employed a final TPTZ concentration of 96 μM at a pH of 4.6 $\pm$ 0.1 after addition of the buffer and the precipitation effect described might not be encountered with their method.

The absorbance of the iron(II) - TPTZ complex can be measured spectrophotometrically within a few minutes of adding the reagents[1–3] and the absorbance is reported to be stable for several hours[1–4]. It is recommended that, if the absorbance is monitored over a long period of time, an uncombined TPTZ concentration of greater than 90 μM should be avoided at pH 4.5.

This investigation was initiated during a study supported by the Centre Européen d'Etude des Polyphosphates E.V. at the Ferry House laboratory of the Freshwater Biological Association, Ambleside, Cumbria, and completed whilst the author held the Richard Claude Mankin Scholarship as a research fellow at the University of Sydney.

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Spectrophotometric Determination of Trace Amounts of Iron(III) with 2-(5-Chloro-2-pyridylazo)-5-diethylaminophenol

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Keywords: *Iron determination*; *spectrophotometry*; 2-(5-*chloro*-2-*pyridylazo*)-5-*diethylaminophenol*

2-(5-Chloro-2-pyridylazo)-5-diethylaminophenol (5-Cl-PADAP) has been used as a chromogenic reagent for spectrophotometric determination of lead[1] and cobalt[2] with satisfactory results because of its high sensitivity and selectivity. During the studies on the spectrophotometric determination of cobalt, we noticed that 5-Cl-PADAP gave a bright blue - purple colour with iron(III) at pH 3–6. On the basis of this new colour reaction, a method has been developed for the determination of iron(III).

The coloured complex exhibits two absorption peaks, at 545 nm and 605 nm. Beer's law is obeyed in the range 0–20 μg of iron(III) per 25 ml of final solution. The colour system is stable for 24 h and is unaffected even by the presence of a large number of foreign ions. The proposed method is highly sensitive, rapid and accurate. It has been applied to the determination of iron in aluminium and magnesium metals and their alloys as well as some products of the chemical industry used for the manufacture of electronic optical glass.

Experimental

Apparatus

Absorption spectra were obtained with a Zeiss Specord ultraviolet - visible double-beam, recording spectrophotometer. For measurements at a single wavelength, a Model 721 spectro-

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photometer was employed. pH measurements were made with a Lei-ci, Model 25, direct-reading pH meter with a glass - calomel electrode assembly.

Reagents

All chemicals were of analytical-reagent grade.

Standard iron solution. Dissolve 0.4317 g of ammonium iron(III) sulphate in distilled water, add 15 ml of hydrochloric acid (1 + 1) and dilute to 500 ml with distilled water. This solution contains 0.1 mg ml^{-1} of iron. Prepare a 10.0 μg ml^{-1} solution by dilution with distilled water.

5-Cl-PADAP solution, 0.02% m/V in ethanol. 5-Cl-PADAP, supplied by the Institute of Environmental Chemistry, Academica Sinica, Beijing (Peking), China, can be prepared[3,4] by coupling 3-diethylaminophenol (0.082 mol) with 5-chloro-2-pyridyldiazoate (0.15 mol) and recrystallising the product from ethanol.

Sodium acetate buffer, pH 3.6. Dissolve 8 g of sodium acetate ($NaC_2H_3O_2.3H_2O$) in distilled water, add 134 ml of 6 M acetic acid and dilute to 500 ml with water.

Recommended Procedures

Into a 25-ml calibrated flask, pipette an aliquot of the sample containing not more than 20 μg of iron and add with swirling 2 ml of sodium acetate buffer (pH 3.6), 5 ml of ethanol and 2 ml of 5-Cl-PADAP solution. Dilute to volume with distilled water and mix well. Measure the absorbance at 605 nm in a 1-cm cell against a reagent blank after 30 min.

Procedure for determination of iron in aluminium and magnesium metals and their alloys

Weigh 0.5 g of the sample, prepare the sample solution by the appropriate method[5] and dilute to 100 ml with distilled water in a calibrated flask. An aliquot of 1–5 ml of this solution is taken for analysis as described above.

Procedure for determination of iron in products of the chemical industry

For nitrate and carbonate salts and boric acid samples, weigh 2–5 g of the sample, dissolve it in 5 ml of hydrochloric acid (1 + 1) and dilute with distilled water to 50 ml in a calibrated flask. An aliquot of 1–5 ml of this solution is taken for analysis as described above.

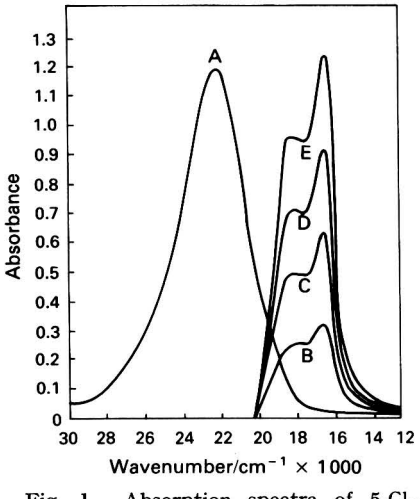

Fig. 1. Absorption spectra of 5-Cl-PADAP (R represents 5-Cl-PADAP in ethanol) and its iron complex, pH 3.6, 1-cm cell. A, 0.02% R against water; B, 5 μg of Fe(III) against R; C, 10 μg of Fe(III) against R; D, 15 μg of Fe(III) against R; and E, 20 μg of Fe(III) against R.

For lead tetroxide (red lead, Pb_3O_4) and quartz, weigh 2–5 g of the sample, prepare the sample solution in the usual way[6] and dilute it to 50 ml with distilled water in a calibrated flask. An aliquot of 1–5 ml of this solution is taken for analysis as described above.

Results and Discussion

Absorption Spectra

The absorption spectra of 5-Cl-PADAP and its iron(III) complex at pH 3.6 in water - ethanol medium are shown in Fig. 1. The iron(III) complex exhibits two absorption peaks, at 545 nm and 605 nm, whereas that of the reagent is at 450 nm. At 605 nm the reagent shows no absorption, and all measurements are therefore made at this wavelength.

Effect of Variables

Effect of pH

The pH of the reaction mixture was varied from 1.0 to 7.0, and the absorbance was found to be maximum in the range 3.0–6.0. Hence, all studies were carried out at pH 3.6 with sodium acetate buffer. The effect of pH on colour development is shown in Fig. 2.

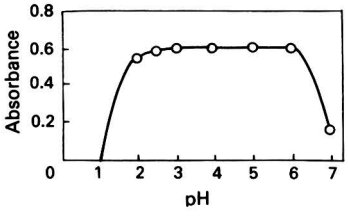

Fig. 2. Effect of pH on absorbance.

Effect of reagent concentration

The absorbance values at pH 3.6 of a water - ethanol solution containing 10 μg of iron and increasing amounts of 5-Cl-PADAP showed that the absorbance remained constant after addition of 1.5 ml of the reagent. Therefore, 2.0 ml of the reagent solution were used in all studies.

Role of ethanol

Because some precipitate was observed after the complex formation, ethanol was added to increase the solubilities both of the reagent and the complex and also the stability of the complex. The addition of ethanol in amounts from 2 to 10 ml gave identical absorbance values. Hence, 5 ml of ethanol was preferred.

Time for the formation of the complex and stability of the complex

The formation of the coloured complex of iron(III) with 5-Cl-PADAP is instantaneous. However, a 20-min standing period was allowed for equilibration. Measurement of the absorbance was therefore carried out 30 min after the addition of 5-Cl-PADAP and thereafter the colour of the complex remained virtually constant for 24 h.

Adherence to Beer's Law and Sensitivity

A series of standard iron solutions were prepared and the absorbance of each was measured and plotted against concentration. From 0 to 20 μg of iron(III) there is a linear relationship between absorbance and concentration. From this straight line, the average molar absorptivity (ϵ) of the iron complex was calculated as 8.82×10^4 l mol^{-1} cm^{-1} and Sandell's sensitivity (based on an absorbance of 0.001 unit) of the colour reaction was 0.00063 μg cm^{-2}. Hence it is evident that the method reported here is more sensitive than that involving pyridylazonaphthol (PAN) ($\epsilon = 1.6 \times 10^4$ l mol^{-1} cm^{-1}) or pyridylazoresorcinol (PAR) ($\epsilon = 4.2 \times 10^4$ l mol^{-1} cm^{-1}).[7]

TABLE I

TOLERANCE LIMITS IN THE DETERMINATION OF 10 μg PER 25 ml OF IRON(III) WITH 5-Cl-PADAP

Ions tolerated	Tolerance limit/ μg per 25 ml
K^+, Na^+, NH_4^+, NO_3^-, Cl^-	5×10^4
Cd^{2+}	3×10^4
Mn^{2+}, SO_4^{2-}	1×10^4
Li^+, Be^{2+}, Mg^{2+}, Al^{3+}, SiO_3^{3-}, Br^-	1×10^3
PO_4^{3-}	500
Ag^+, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Hg^{2+}, Y^{3+}, La^{3+}, Pb^{2+}, Cr^{3+}, Mo^{6+}	100
Cr^{6+}	85
Cu^{2+}, Co^{2+}, Ni^{2+}	2

TABLE II

RECOVERY OF ADDED IRON FROM PRODUCTS OF THE CHEMICAL INDUSTRY

Sample	Original iron content, %	Iron added, %	Iron found, %	Recovery of added iron, %
Potassium nitrate	0.0023	0.0005	0.00284	108
	0.0023	0.001	0.0035	105
Barium carbonate	0.0013	0.0005	0.00181	102
	0.0013	0.001	0.00233	102.5
Red lead	0.0034	0.0005	0.00389	97
	0.0034	0.001	0.00436	96

TABLE III

RESULTS OF REPLICATE ANALYSES

Sample	Mean iron content (eight determinations), %	Standard deviation, %	Coefficient of variation, %
Boric acid	0.0020	0.00015	0.075
Red lead	0.0013	0.00013	0.10
Quartz	0.0034	0.00013	0.038

TABLE IV

COMPARISON OF RESULTS BETWEEN 1,10-PHENANTHROLINE AND 5-Cl-PADAP METHODS OF DETERMINING IRON IN MISCELLANEOUS SAMPLES

Sample	Iron content, %	
	By 1,10-phenanthroline method	By 5-Cl-PADAP method
Aluminium metal	0.060	0.062
Magnesium metal	0.060	0.061
Magnesium metal	0.031	0.030
Aluminium alloy	0.044	0.050
Magnesium alloy	0.014	0.013
Potassium carbonate	0.0070	0.0068
Sodium carbonate	0.0044	0.0041
Barium carbonate	0.0037	0.0034
Potassium nitrate	0.0024	0.0023
Barium nitrate	0.0014	0.0013
Boric acid	0.0022	0.0020
Red lead	0.0015	0.0013
Quartz	0.0035	0.0034

Effect of Foreign Ions

The effect of various amounts of 30 foreign ions on the determination of 10 μg of iron(III) per 25 ml of solution was examined under the experimental conditions used. The tolerance limits showed that iron(III) could be determined in the presence of a large number of ions (Table I). Hence, the selectivity of the present method is good.

Composition of the Complex

The composition of the complex was determined by the continuous variation method[8] (Fig. 3) and the molar-ratio method[9] (Fig. 4). A ratio of iron(III) to 5-Cl-PADAP of 1:2 in the complex molecule was obtained by both methods.

Precision and Accuracy

Some typical results obtained for the recovery of known amounts of iron added to three samples and 8-fold replicate determinations on each of the three samples are shown in Tables II and III, indicating that the method is reproducible and accurate. The results of analysis in comparison with the 1,10-phenanthroline method[10] are given in Table IV, showing the satisfactory nature of the procedure.

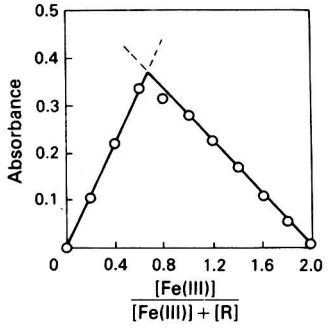

Fig. 3. Determination of the composition of Fe(III) - 5-Cl-PADAP (R) complex by continuous variation method. [Fe(III)] + [R] = 8.0 × 10^{-6} M.

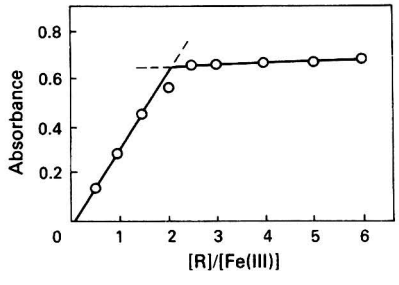

Fig. 4. Determination of the composition of Fe(III) - 5-Cl-PADAP (R) complex by molar ratio method.

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Spectrophotometric Determination of Micro-amounts of Chromate in the Presence of Iron(III), Chromium(III) and Other Ions

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Keywords: *Chromium(VI) determination*; *spectrophotometry*; *iron(II) - FerroZine complex*

Chromium is used not only in industrial processes but in its trivalent oxidation state is also an essential nutrient. However, chromate and dichromate are toxic and must be monitored.

Micro-amounts of chromium(VI) have been determined not only spectrophotometrically using diphenylcarbazide,[1–5] varamine blue,[6] alizarin,[7] pyridine-2,4,6-tricarboxylic acid,[8] triethylenetetraaminehexaacetic acid (TTHA),[9] crystal violet[10] and α-naphthyl[11] but also polarographically,[12,13] potentiometrically[14,15] and voltammetrically.[16] Most of these methods are subject to some interference from ions such as iron(III), tungsten(VI), vanadium(V) and chromium(III).

An indirect method for the determination of chromium(VI) has been developed and is described here. Chromium(VI) is reduced to chromium(III) in the presence of iron(II) and the excess of iron(II) is then determined as a complex with FerroZine [monosodium 3-(2-pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine monohydrate] that has a molar absorptivity of 28000 l mol^{-1} cm^{-1}. In this manner, chromium(VI) at the parts per million level has been determined by measuring the decrease in the absorbance of the iron(II) - FerroZine complex at 562 nm. Relatively large amounts of iron(III), chromium(III) and vanadium(V) can be tolerated. This method is fast and sensitive; however, the range of chromium(VI) concentrations must be established by trial and error.

Experimental

Apparatus

A Cary 14 spectrophotometer was used for the absorbance measurements. The pH of the solutions was determined using an Orion pH meter.

Reagents

All reagents were of analytical-reagent grade and were used without further purification. Stock solutions of chromium(VI), vanadium(V), nickel(II), cobalt(II), chromium(III) and manganese(VII) were prepared by dissolving weighed amounts of the appropriate compounds.

Approximately 40 mg of ammonium iron(II) sulphate (Mallinckrodt Inc., analytical grade) were dissolved in 100 ml of 0.1 N sulphuric acid. Fresh solutions of iron(II) were prepared daily. Approximately 0.1 M chloroacetate was prepared by dissolving 9.5 g of monochloroacetic acid in 600 ml of water, adjusting the pH to 5.8 using 3.0 M sodium hydroxide solution and then diluting to 1 l. A 0.012 M stock solution of FerroZine (Hach Chemical Co.) was prepared by dissolving 0.6125 g in 100 ml of de-ionised water.

Procedure

Determination of chromium(VI) alone and in the presence of non-interfering ions

Standard chromium(VI) solutions were prepared by diluting 1 ml of 0.010 M chromium(VI) stock solution to 100 ml with de-ionised water. Polyethylene flasks were used to store dilute solutions and to prevent adsorption of chromate. Aliquots of this solution (0.5–6.0 ml) were transferred into 50-ml polyethylene calibrated flasks for standardisation. Exactly 2 ml of a 0.001 M iron(II) solution in 0.1 N sulphuric acid were added to each flask followed by 1.0 ml of 0.012 M FerroZine solution. After 2 min, 6.0 ml of chloroacetate buffer solution were added

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and the contents were diluted to volume. The residual absorbance was measured at 562 nm after 3 min using 1-cm cells. The absorbance (ΔA) due to the reaction of chromium(VI) with iron(II) was obtained by taking the difference between the absorbance values of iron(II) alone and in the presence of chromium(VI).

In the presence of vanadium(V), chromium(III), cobalt(II), nickel(II) and copper (II), the procedure followed is identical with that given above except that the amount of FerroZine solution was increased from 1 to 2 ml, and the initial pH of the synthetic samples was adjusted with dilute sulphuric acid or sodium hydrogen carbonate solution to be in the range 2.0–3.0, prior to the addition of iron(II).

Determinations of chromium(VI) in the presence of permanganate

Suitable aliquots of chromium(VI) and permanganate solutions were transferred into 40-ml beakers. Solutions were acidified with 5 drops of 0.1 N sulphuric acid and heated. Then to each beaker 5 drops of 0.1 M sodium azide solutions were added and the solutions were boiled gently until the colour of permanganate disappeared. Heating was continued for an additional 3 min in a well ventilated hood until all of the hydrogen azide had been eliminated. After cooling the solutions to room temperature, 2.00 ml of iron(II) and 2 ml of FerroZine solutions were added to each beaker. The pH was adjusted by adding 6.0 ml of chloroacetate buffer solution and the contents were transferred into 50-ml calibrated flasks and diluted to volume.

Determination of chromium(VI) in the unknown samples was carried out by first establishing the amount of sample or volume of the aliquot to contain 5–30 μg of chromium(VI) so that the absorbance difference (ΔA) between the absorbance due to iron(II) alone and that due to iron(II) in the presence of chromium(VI) fell in the range 0.15–0.90 absorbance unit. Once the approximate amount of chromium(VI) had been determined, the sample size was adjusted to the optimum value. Also, in some instances, a known amount of chromium(VI) was added and the amount of chromium(VI) was calculated using the following equation:

$$\Delta A_{\text{unk.}}/\Delta A_{\text{unk.}+\text{std.}} = [\text{Cr(VI)}]_{\text{unk.}}/[\text{Cr(VI)}]_{\text{unk.}+\text{std.}}$$

Results

Determination of chromate alone with iron(II) provides information concerning not only the optimum conditions to be used but also the apparent molar absorptivity for the reaction and and the sensitivity of the method. Results for the difference in absorbanee values (ΔA) between samples containing iron(II) alone and in the presence of chromate are summarised in Table I.

Analyses of chromate samples alone give a value of 83200 l mol^{-1} cm^{-1} for the apparent molar absorptivity as compared with the theoretical value of 84000 l mol^{-1} cm^{-1} for the three-electron transfer by chromium(VI). Further, it establishes that the concentration of chromium(VI) in the unknown solutions can be determined in the range 5–30 μg when using 50-ml volumes for the absorbance measurement.

TABLE I

ABSORBANCE DIFFERENCES (ΔA) AS A FUNCTION OF CHROMIUM(VI) CONCENTRATION

Amount of chromium(VI)/μg per 50 ml		
Taken	Found*	Absorbance difference (ΔA)†
5.20	5.28 ± 0.15	0.169
10.40	10.4 ± 0.20	0.334
15.60	15.3 ± 0.15	0.489
20.79	21.0 ± 0.30	0.673
26.00	25.7 ± 0.41	0.823

* Amount of chromium(VI) found is calculated by using the apparent molar absorptivity of 83200 l mol^{-1} cm^{-1} and the expression

$$(1000\ \Delta A \times 50 \times 51.99)/83200 = 31.24 \times \Delta A$$

† The absorbance difference (ΔA) is the difference in absorbance values for samples containing iron(II) alone and those containing iron(II) in the presence of chromium(VI). The reported values of ΔA are the averages of four determinations.

Results for the synthetic samples, summarised in Table II, indicate that chromium(VI) can be determined with a relative precision of better than 2%, provided that the concentrations of cobalt(II), nickel(II) and permanganate are not greater than approximately 0.2 p.p.m. Vanadium(V) can be present at levels as high as 1 p.p.m. and iron(III) and chromium(III) can be present at levels as high as those of the iron(II) added.

Discussion

Determination of chromium(VI) is achieved indirectly through its reaction with an excess of iron(II):

$$Cr_2O_7^{2-} + 6Fe^{2+} + 14H^+ \xrightarrow{\text{pH 1–2}} 2Cr^{3+} + 6Fe^{3+} + 7H_2O$$

followed by the chelation of the iron(II) with FerroZine (Ferz):

$$Fe^{2+} + 3Ferz^{2-} \xrightarrow{\text{pH 3.1–3.5}} Fe(Ferz)_3^{4-}$$

and measurement of the resulting absorbance at 562 nm.

Reduction of chromium(VI) proceeds readily in acidic solutions, so the pH must not be greater than 2.0 or the reaction will be slow and not quantitative. Normally, the desired pH is achieved by using a standard iron(II) solution that has been prepared in 0.1 N sulphuric acid, provided that the solutions of unknown chromate concentration are neutral or have pH values above 2.0. If the samples of chromate are basic, they are acidified by addition of a few drops of dilute sulphuric acid. Under acidic conditions, the reduction of chromate is complete in less than 1 min. In the presence of vanadium(V), the pH of the solution should not be less than 1, otherwise some vanadium(V) will react with iron(II) and will yield higher values for the chromium(VI) concentrations. The formation of the iron(II) - FerroZine complex requires pHs higher than 3.0. In this determination the pH is kept in the range 3.0–3.5 in order to prevent the formation of an insoluble hydrated iron(III) oxide, which can be a problem with high iron(III) concentrations. The interference of iron(III) can be overcome either by keeping the pH below 3.5 or by the addition of ammonium fluoride or sodium dihydrogen phosphate. The colour of the iron(II) - FerroZine complex is stable and requires no special timing before the measurement. Thus, the concentration of these potentially interfering ions should not be greater than that of chromium(VI) and in their presence it is advisable to add an additional 1 ml of FerroZine solution.

Permanganate interferes seriously and must be reduced preferentially using sodium azide in a hot acidic solution. The excess of hydrogen azide is eliminated by heating the solution in a well ventilated hood. In general, the results for chromium are lower than expected. Thus, the amount of permanganate that can be tolerated must not be greater than 0.2 p.p.m.

TABLE II

ANALYSIS OF SYNTHETIC CHROMATE SAMPLES IN THE PRESENCE OF OTHER IONS

Sample No.	Ions present in sample *	Amount of chromium(VI)/ μg per 50 ml: Taken	Found†	Absorbance difference (ΔA)
1	V(V) and Cr(VI)	5.20	5.31	0.170
		26.0	25.6	0.820
2	Cr(III), Fe(III) and Cr(VI)	5.20	5.34	0.171
		26.0	25.5	0.815
3	Mn(VII) and Cr(VI)	5.20	5.16	0.165
		26.0	25.4	0.814
4	Mn(VII), Ni(II), Cr(III), V(V), Fe(III), Co(II) and Cr(VI)	5.20	4.85	0.155
		26.0	25.2	0.805

* Amounts of ions present: sample 1, 25 μg of V(V) (as VO_3^-) and varying amounts of Cr(VI); sample 2, 52 μg of Cr(III), 56 μg of Fe(III) and varying amounts of Cr(VI); sample 3, 5.5 μg of Mn(VII) (as MnO_4^-) and varying amounts of Cr(VI); and sample 4, 5.1 μg of V(V), 5.2 μg of Cr(III), 5 μg of Ni(II), 5 μg of Mn(VII) (as MnO_4^-), 5.6 μg of Fe(III) and 5 μg of Co(II).

† Amount of Cr(VI) is calculated by using the apparent molar absorptivity of 83 200 l mol^{-1} cm^{-1} and the expression 31.24 × ΔA. All determinations were carried out in duplicate.

Vanadium(V) can be tolerated in amounts of up to five times that of chromium(VI). It is essential to keep the pH of the solution in the range 1–2. At pH values less than 1, some vanadium(V) will react with iron(II), and at pHs higher than 2 the reaction of chromium(VI) is incomplete and the results are low. The relative precision for the absorbance measurement remains in the order of 1.7%. However, in the presence of cobalt(II) and nickel(II), results for dichromate are low because these ions form FerroZine complexes that contribute towards the absorbance of the solution by a small amount.

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Dithiooxamide as a Reagent for the Detection and Spectrophotometric Determination of Chloral Hydrate in Alcoholic Beverages

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Keywords: *Chloral hydrate determination*; *dithiooxamide*; *spectrophotometry*; *alcoholic beverages*

Chloral hydrate serves as an excellent hypnotic when administered in therapeutic doses, making it subject to drug abuse. As it has a very pungent odour and bitter taste it is commonly added illicitly to alcoholic beverages, which increases their potency.[1] Therefore, methods for its detection and determination have been a subject of forensic interest in recent years.

A method for the detection and determination of chloral hydrate using thiobarbituric acid as a reagent has been reported from our laboratories.[2–4] Although it is sensitive and specific for chloral hydrate it requires very rigid temperature control (32 °C for 100 min). While studying various reagents for use in the detection of chloral hydrate, we found that heating with dithiooxamide in an alkaline medium over a boiling water-bath for 5 min gave a pink complex ($\lambda_{max.}$ 550 nm). This reaction is described in this paper for the determination of chloral hydrate.

Experimental

Reagents

Chloral hydrate stock solution. Dissolve 1.00 g of chloral hydrate in distilled water and dilute the solution to 1 l.

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Dithiooxamide reagent. Dissolve 0.17 g of dithiooxamide (BDH) in 100 ml of rectified spirit.
Hydrochloric acid, 1 M.
Ammonia solution, 3 M.
Orthophosphoric acid, 0.1 M.

Apparatus

Spectrophotometer. Carl Zeiss Specord with 10-mm cells and Spekol spectrophotometers.

Procedures

Colour development

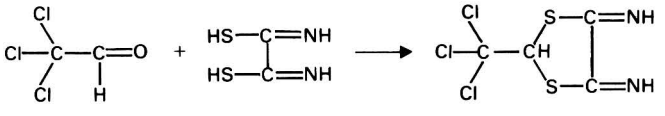
Accurately transfer 4 ml of chloral hydrate standard solution into a test-tube. Add 1.5 ml of dithiooxamide reagent and 2.5 ml of hydrochloric acid followed by 2 ml of ammonia solution, shaking intermittently. Keep the test-tube in a water-bath (50–80 °C) for 5 min, then remove it and after 30 min measure the absorbance of the pink colour at 550 nm against a reagent blank. Standards in the range 10–70 μg ml^{-1} were treated, together with a reagent blank, and the absorbances were read at 550 nm using the Spekol spectrophotometer. If a sample solution containing chloral hydrate has a low pH it is necessary to adjust it to 6.8 by the addition of ammonia solution before the colour will develop.

Application to alcoholic beverages

A 100-ml sample of an alcoholic beverage was used, to which 2–6 mg of chloral hydrate were added for recovery studies. Each sample was distilled, after acidifying with 5 ml of orthophosphoric acid, and 50 ml of distillate were collected, an aliquot of which was then treated with dithiooxamide, as reported above.

Results and Discussion

Dithiooxamide is used in a spot-test for the detection of metal ions, *e.g.*, copper, cobalt and nickel.[5] Dithiooxamide in its tautomeric dithiol (or diimido) form condenses with chloral hydrate but not with aldehydes such as acetaldehyde, possibly because of the strong inductive effect of the chlorine atoms.



When acetaldehyde, formaldehyde, trichloroacetic acid, carbon tetrachloride and chloroform were treated similarly with dithiooaxamide, they did not form any coloured compounds. It can also be noted that the formation of a coloured complex between chloral hydrate and dithiooxamide is dependent upon the pH, *i.e.*, above 6.8, of the solution, which can be achieved by adjusting the proportion of acid and alkali as given in the procedure.

The coloured complex developed with chloral hydrate exhibits maximum absorption at 550 nm and obeys Beer's law over a wide concentration range (10–70 μg ml^{-1}).

Recovery studies were carried out on various pure alcoholic beverages by adding known amounts (2–6 mg) of chloral hydrate before the distillation stage and chloral hydrate was then determined as described above. From the data given in Table I it can be seen that the recovery is in the range 96.6–104%.

The proposed method for the determination of chloral hydrate when in routine use is sensitive, specific and rapid.

The authors are grateful to Dr. B. N. Mattoo (Director, Forensic Science Laboratory) for his encouragement.

TABLE I

RECOVERY OF ADDED CHLORAL HYDRATE FROM ALCOHOLIC BEVERAGES BY DISTILLATION

Sample (100 ml)	Chloral hydrate content/mg		
	Added	Recovered*	Recovery, %
Rum	2	2.08	104
	3	2.9	96.6
	4	3.98	99.5
			Mean: 100
Brandy	2	1.98	99
	3	3.05	101.7
	4	3.95	98.7
			Mean: 99.8
Whisky	3	3	100
Whisky	4	4.02	100.5
	5	4.94	98.8
			Mean: 99.8
Toddy †	4	3.98	99.5
	5	4.95	99
	6	5.52	92
			Mean: 96.8

* Average of three determinations.
† Fermented juice obtained from palm trees.

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Communications

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.

Sample Re-radiation Effects in the Quantitative Analysis of Crystalline Silica in Foundry Samples by Infrared Spectrophotometry

Keywords: *Crystalline silica*; *quartz*; *cristobalite*; *tridymite*; *infrared spectrophotometry*

The two principal methods currently employed in the quantitative determination of crystalline silica are X-ray diffraction (XRD) and infrared spectrophotometry, both of which are capable of identifying free silica in the presence of silicates.[1] XRD analysis, although well established,[2–11] is prohibitively expensive for many laboratories. Consequently, relatively inexpensive dispersive infrared spectrophotometers are being increasingly used to analyse crystalline silica, by either the direct[12–14] or the potassium bromide disc[15–19] method. In the former method, the membrane filter on which the sampled dust is deposited is placed directly in the infrared beam, whereas in the latter the sampled dust is recovered from the filter by incineration and mixed with potassium bromide, from which a disc is prepared for infrared analysis.

In either instance infrared source radiation, after suitable collimation, passes through the sample where certain wavelengths are absorbed owing to transitions between the vibrational energy levels of the molecules comprising the sample. Although many compounds or minerals that can occur with crystalline silica in mining and industrial environments have well defined, specific absorptions within the mid-infrared region, several possess extensive absorption continuums, especially in the higher energy region above 1 500 cm^{-1}. Compounds such as graphite and magnetite, for example, which are commonly found with crystalline silica in foundry atmospheres, act as black-body radiators within this region of the electromagnetic spectrum. In this instance, although the incineration stage of the potassium bromide disc method will readily oxidise graphite, unchanged magnetite will remain in the ashed sample.

Having absorbed substantial amounts of radiant energy, such compounds will rapidly return to their vibrational ground states by collisional deactivation and radiative processes. The infrared detector will therefore receive more radiant energy at most lower frequencies than the sample absorption processes would suggest, owing to sample re-radiation effects, which will effectively attenuate any absorption bands of interest. In order to overcome such problems several commercial dispersive infrared spectrophotometers are equipped with an asynchronously double-chopped optical system. However, most inexpensive instruments currently being employed in the analysis of crystalline silica will produce increasingly erroneous results as the true transmission of the sample spectrum base line decreases. We have found up to 75% attenuation in the crystalline silica concentration of certain types of foundry samples using such instruments.

An effective means of reducing sample re-radiation effects to a negligible level in crystalline silica analysis is to incorporate a 9-μm germanium cut on/blocking filter (Specac Ltd., Orpington, Kent) prior to the sample. The mid-infrared spectrum is shown in Fig. 1, together with the base-

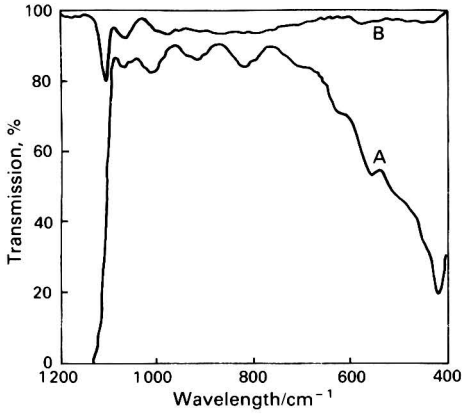

Fig. 1. Infrared spectrum of 9-μm germanium cut on/blocking filter: A, single filter in sample beam; and B, additional filter in reference beam.

line spectrum obtained by inserting an identical filter in the reference beam. The maximum transmittance in the cut on region is >90%, with <0.001% transmittance above 1150 cm^{-1}. A typical infrared spectrum of respirable-size airborne foundry dust obtained by the direct infrared method is shown in Fig. 2. With the inclusion of a pair of cut on/blocking filters fitted prior to the sample and reference membrane filters, the infrared absorbance of the characteristic α-quartz doublet at 800 and 780 cm^{-1} has been doubled (Fig. 3).

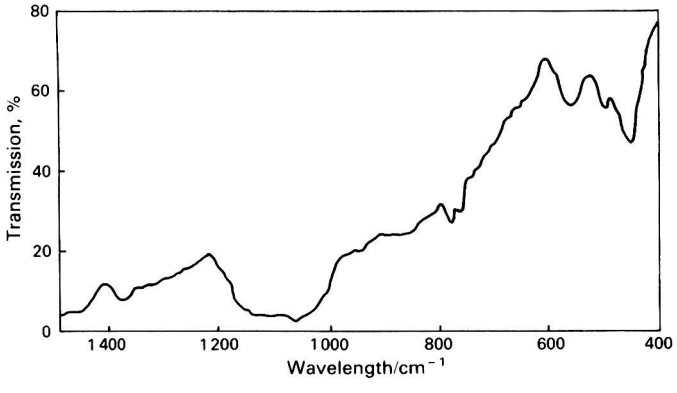

Fig. 2. Typical infrared spectrum of airborne foundry dust.

The results obtained using a pair of cut on/blocking filters with a simple dispersive infrared spectrophotometer have been compared with those obtained with an asynchronously double-chopped instrument and XRD analysis on the same membrane filters for a wide range of samples. In all instances where interferences from mineral absorptions were absent, the correlation between the three sets of results was within 10%, indicating that sample re-radiation effects had been eliminated. Fig. 4 shows the very good agreement obtained for a batch of black foundry samples

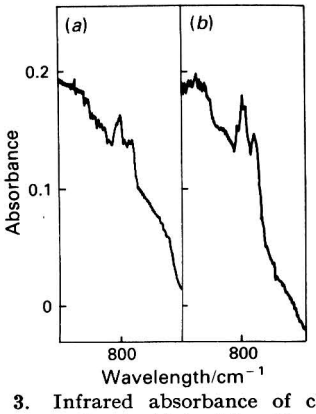

Fig. 3. Infrared absorbance of characteristic α-quartz doublet: (*a*) without cut on/blocking filter; and (*b*) with filter in both sample and reference beams.

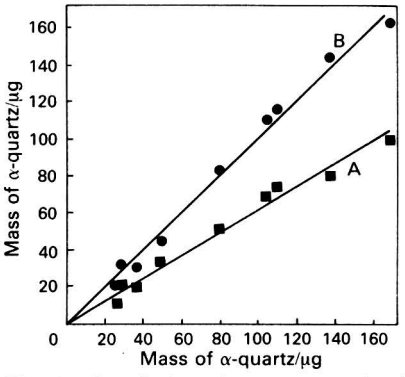

Fig. 4. Correlation of α-quartz results for a batch of black foundry samples between asynchronously double-chopped (*x*-axis) and conventional dispersive (*y*-axis) instruments; A, without cut on/blocking filters in latter instrument; and B, with filters.

between asynchronously double-chopped and conventional dispersive instruments when cut on/blocking filters were fitted to the latter instrument.

We thank Mr. D. J. Butler for valuable assistance in the analysis of the crystalline silica samples.

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A Toluidine Blue Stain Mountant for the Microscopy of Comminuted Meat Products

Keywords: Microscopy; single-stage staining; meat tissues; wheat gluten; soya protein determination

In two earlier papers[1,2] the use of a one-step metachromatic staining method using toluidine blue was recommended. An advantage of this method in quantitative microscopy is that any loss of section constituents is avoided. In both qualitative and quantitative work the wide range of

colours rapidly obtained on microsections enables soya products and wheat gluten to be readily distinguished from muscle fibres and connective tissue.

Since the publication of the staining method, the manufacture of the aqueous mountant (Gurr's Uvak) recommended as the vehicle for toluidine blue has ceased and experiments with both commercial mountants and textbook aqueous mountants[3] have given unsatisfactory results.

The proposed staining medium has the advantage of using known constituents, it is simple to prepare and use and the differential staining achieved is marginally superior to that given by the previously published formulation.

Experimental

Staining Medium

This consists of a 0.2% solution of toluidine blue in 30% aqueous glycerol containing 0.66% of phenol. To gain full differential staining, which depends on the presence of dyestuff polymers, the sequence of solution preparation is important. Dissolve 0.29 g of toluidine blue (CI 52040) in 100 ml of distilled water. Add 30 ml of glycerol to 70 ml of the aqueous toluidine blue, mix and add 0.66 g of phenol. Allow the mixture to stand overnight.

Materials Tested

The materials tested were 10-μm cryostat sections prepared from fresh minced beef, raw commercial sausage, soya grits, soya protein isolate, texturised soya protein (TSP) and commercial wheat gluten.

Staining Procedure

Using a dropper bottle, place one or two drops of the staining medium on the section so that it is completely covered. Leave for 1 min and then cover with a cover-slip. Allow a delay of 5 min to ensure complete staining, remove excess of staining medium by careful blotting and examine.

Results and Discussion

The appearance of the stained sections was substantially the same as previously reported.[1,2] The muscle fibres in the minced meat appeared pale blue with muscle cell nuclei red-violet. The muscle fibres in the commercial sausage appeared a pale purple colour, owing to the presence of inorganic phosphates in the sausage, which affects the polymerisation of the toluidine blue and is also seen with the toluidine blue in Uvak staining medium. Collagenous tissue in the fresh meat was coloured pale pink, and the rind present in the sausage appeared a much paler and more lilac colour, owing to the extraction of metachromatic mucopolysaccharides from the rind during processing. Elastic tissue fibres appeared the same pale blue-green colour in both connective tissue and processed rind. The soya protein in the grits, isolate and TSP appeared a dark purple blue, and the soya cell walls in the grits and TSP stained a deep pink colour. Commercial wheat gluten coloured a pale blue-green.

In addition to being independent of a commercial mountant of undisclosed constitution, the staining medium described has two advantages over the toluidine blue in Uvak medium: it does not need the 10-d "ripening" period, being ready to use within 24 h, and the stained sections, though not permanent, keep better. Staining is almost unchanged overnight and useful colour contrast persists for 1–2 d.

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Book Reviews

MICROBIOLOGICAL APPLICATIONS OF GAS CHROMATOGRAPHY. By D. B. DRUCKER. Pp. viii + 478. Cambridge University Press. 1981. Price £45. ISBN 0 521 22365 2.

This book is an interesting combination of chemistry and microbiology and is published at a time when chemical techniques are being used more and more in the biological sciences. Sufficient microbiology is included to allow the chemist to appreciate the inherent difficulties of analysing biological materials, and sufficient chemistry is included to allow the microbiologist to appreciate and understand the principles of gas chromatography. Thus, the volume will serve as a practical guide for the microbiologist using this chemical technique and for the chemist confronted with biological samples.

The book is logically written, beginning with a detailed account of the theory of gas chromatography. Descriptions of the various components of gas chromatographs are included, together with details of the most appropriate system to use for particular types of applications. The author then concentrates on the applications of the technique, with chapters on the analysis of fermentation products, the detection of microorganisms and the analysis of metabolites. The growth and metabolism of microorganisms is described with detailed methods for the analysis of neutral, basic and acidic fermentation end-products together with headspace gas analysis and the identification of anaerobes. The advantages of gas chromatography over the classical microbiological procedures for the detection of microbes are promulgated and the development of the methods used in lunar and planetary exploration described. Methods are then detailed for the analysis of food samples and clinical specimens such as cerebrospinal fluid, urine, blood, serum, pleural effusions and pus. The fourth chapter describes the chemistry of microbial cells, the fractionation of cells and the subsequent analysis of cellular components such as lipids, proteins, nucleic acids, polysaccharides, teichoic acids and peptidoglycans. Methods for the analysis of specific metabolites, intermediates and specific enzymes are also described, together with applications particularly appropriate to industry. The latter include the analysis of alcoholic beverages such as beer and wine, various dairy products, fish, vaccines, antibiotics and mycotoxins.

The penultimate chapter is devoted to the rather specialised field of pyrolysis - gas - liquid chromatography. The instrumentation required is described, together with applications of the technique in taxonomy, detection and characterisation of microbial products. The final chapter describes the problems of handling what can be vast amounts of information generated in a short time. Methods for handling the data are suggested, together with systems of analysis, interpretation and usage of the information obtained.

The book is very well written and has clear and concise diagrams and an abundance of figures and tables which amply illustrate the text and provide examples of sample results. The literature is continually referenced throughout and 800 papers are cited. There is a wealth of detailed practical information that has not been accumulated in this form in any other text, and the book should be welcomed by workers in both research and industry.

R. F. COSGROVE

ANALYSIS OF PESTICIDES. Edited by H. ANSON MOYE. *Chemical Analysis: A Series of Monographs on Analytical Chemistry and its Applications, Volume* 58. Pp. x + 467. John Wiley. 1981. Price £25.75. ISBN 0 471 05461 5.

The book contains ten chapters by different authors on various aspects of residue or trace analytical science as applied to the problems of increasing use of synthetic chemicals in agriculture. Its 467 pages are excellently produced, easy to read and remarkably free from errors. I looked at it critically as there are many reviews of the literature available, some of which are not very helpful to the residue chemist.

Environmental concern about pollution and the resulting legislation have led in recent years to a massive advance in analytical technology. This is amply reflected in this book. Chapters 1 and 2 give an excellent account of column and detector technology in gas chromatography, and Chapter 3 deals with the still very useful art of thin-layer chromatography. I was pleased to see that in this chapter the authors had not forgotten the problem of extractability of "weathered" residues, which detracts from the value of some earlier work in residue analysis. Chapter 4 is an especially valuable

account of the most exciting analytical development in recent years, high-performance liquid chromatography, and the author is to be congratulated on his very readable style.

Chlorinated hydrocarbons are still of very great interest to environmental scientists, particularly with the recognition of the widespread dissemination of polyhalogenated compounds originating from a wide spectrum of industrial activities. I thought that Chapter 5 could have elaborated more fully on this point. Chapter 6, in dealing with the analysis of acidic herbicides, is exceptionally valuable. It has the authentic authority of "workers at the coal face," whose expertise has been constantly tested by the demands of their employment.

Chapters 7 and 8 report on the residue analysis of organophosphorus and carbamate agrochemicals, respectively. I thought that these chapters were competently written but without being exciting. I wished at times that the authors had been more selective in their choice of references.

Chapter 9 is a somewhat tentative account of the analytical chemistry of pheromones and chapter 10 summarises the regulatory requirements imposed upon agrochemical manufacturers. I thought the inclusion of this chapter a very good idea in an otherwise technically orientated book. I found Chapter 10 hard to read, but that may reflect more on the complexities of the subject rather than the authors' style. Over all I consider the book to be a valuable addition to the residue Chemist's library and I can confidently recommend it to all analytical scientists who might wish to remain informed about the technology that feeds the environmental debate. D. J. W. BULLOCK

OPTIMISATION IN HPLC. By R. E. KAISER and E. OELRICH. Pp. 278. Dr. Alfred Hüthig Verlag. 1981. Price DM66; $33. ISBN 3 7785 0657 9.

This book is in two more or less independent parts. Part 1 by Dr. R. E. Kaiser is essentially theoretical and deals with physical, chemical and mechanical optimisation of HPLC; Part 2 by Dr. E. Oelrich and co-workers describes the construction and practical operation of a commercial HPLC cassette system.

Part 2 can be reviewed simply. The HPLC cassette system was developed jointly by Dr. Kaiser's Institute for Chromatography and the E. Merck Company in Darmstadt. It has three essential features: firstly the use of 0.27 mm bore syringe tubing (0.5 mm diameter) for all connections, secondly a miniturised PTFE sealing system and thirdly a series of specially designed zero dead volume connectors, T-pieces, etc., which can be coupled together and held in a readily assembled clamping system. The system allows for great versatility in coupling columns and appears to deal in an elegant way with the problem of reducing dead volumes. It is therefore unfortunate that so little evidence is presented that it is actually superior to existing systems. A valuable feature of Part 2 is the detailed descriptions of various column switching, back-flushing and heart-cutting techniques that can be performed with the cassette system, together with real examples of its use. This part will be useful to many liquid chromatographers who are as yet unfamiliar with these techniques which are so widely used in gas chromatography.

The review of Part 1 presents an entirely different problem. Reading Part 1 one is first fascinated, then frustrated and finally forced to the conclusion that the concepts presented are totally unsatisfactory and indeed scientifically unsound.

Initially one is fascinated by the racy style, which criticises without fear or favour both ideas and personalities in HPLC. One then becomes frustrated that polemics obscure the arguments. Rarely has this reviewer come across a worse presentation of what purport to be new scientific principles. Finally, when one has untangled what the author is trying to say one realises that his arguments are scientifically unsound. Dr. Kaiser's first aim is to discredit the current way in which two widely accepted theoretical ideas are used in practice, namely the concept of the height equivalent to a theoretical plate, and the concept of the additivity of variances on the grounds that chromatographers always assume peaks to be Gaussian when in fact they very rarely are. He advocates replacement of this theoretical structure by his "*abt* concept," which he claims has a firm practical foundation. The concept is simple, namely that the peak width, b (measured at any convenient fraction of maximum peak height), is a linear function of the capacity factor, k';

$$b = b_0 + ak'$$

If $b_0 = a$ then the peak width is proportional to the retention time and the plate count, N, is constant. If b_0 exceeds a, N increases with k'—the normal situation when instrumental broadening is important—whereas if b_0 is less than a, N decreases with k'—the ideal situation when there is no

instrumental contribution. It is claimed that a and b_0 are independent of substance and should be determined using a homologous series. The equation permits a simple calculation of peak capacity (misleadingly called separation number). Nobody would quarrel with this, although they might question whether one really obtains a linear relation in practice. However, most chromatographers do not use this kind of arithmetic in any case! What one cannot agree with are the associated statements. All those familiar with basic chromatographic theory will strenuously disagree with the views that "Peak broadening components having instrumental, technical and methodological origins can be eliminated by simple subtraction of width components" (p. 96) and that "b_0 accounts additively for all peak broadening effects external to the column" (p. 97). These statements are simply incorrect and cannot be allowed to stand. They are completely misleading as a guide to the improvement of equipment.

A second peculiarity of this presentation is the method advocated for determining t_m, the dead time of a chromatographic system that is used as the basis for measuring k'. Dr. Kaiser defines t_m correctly as "the residence time of substances in the mobile phase" (p. 102). He then states without any proof or justification that "Dead time is a calculated value which corrects the total retention times, t_{ms}, of homologues such that the resulting logarithms of the net retention times, t_s, show a strict linear correlation with the molecular weight" (p. 109). Linear correlations of log k' with molecular weight are examples of additivity of group free energies and are widely known in thermodynamics as approximations. There is absolutely no justification for converting such an approximation into a law and using it to determine a perfectly well defined quantity t_m.

While this part of the book presents some practical recommendations (which could be given in a few pages) it is most unfortunate that the whole theoretical basis of the recommendations is misconceived and scientifically unsound. It is therefore a hindrance to the advance of science. It is doubly unfortunate that the arguments, such as they are, are so badly presented in a style which is at the same time highly polemical, full of irrelevant asides and bristling with scientific and logical syntactic errors. It is indeed difficult to see how such a book came to be published as it can only bring discredit on its publisher and senior author.

JOHN H. KNOX

MASS SPECTROMETRY OF PRIORITY POLLUTANTS. By BRIAN S. MIDDLEDITCH, STEPHEN R. MISSLER and HARRY B. HINES. Pp. xii + 308. Plenum. 1981. Price $29.50. ISBN 0 306 40505 9.

A consent decree issued by the Environmental Protection Agency (EPA) in the USA named 129 individual substances that were to be monitored and regulated in the environment. Of these compounds, 114 organic "priority pollutants" were required to be analysed by combined gas chromatography - mass spectrometry. Many manufacturers of such instruments took up the challenge and produced equipment specifically designed for monitoring these priority pollutants. Systems are automated so that routine monitoring can be carried out by less highly trained personnel.

Middleditch, Missler and Hines have produced a compilation of mass spectra of the 114 organic priority pollutants designed to aid the identification of these compounds using the relatively basic instruments favoured for this work. The authors claim the book is for the "new breed of mass spectroscopist . . . who merely wishes to identify specific pollutants in effluents." It is their hope that it will "find a place on top of every mass spectrometer used for the analysis of priority pollutants."

The compilation itself begins with a brief introduction, listing the compounds and their EPA classification. The bulk of the book is devoted to a series of entries depicting a bar spectrum, mass listing, classification and other details of each pollutant. Individual bibliographies are also given. Included are spectra for the recommended internal standards. Finally, an appendix contains "Eight Peak Index" style listing by molecular weight, and by base peak, second and third next intense peaks.

In assessing this book, one must ask if it can aid in the identification of specific compounds. The answer must be "yes," but not as a single reference source, and here the book is disappointing. It is solely a compilation of mass spectra. No experimental details are given but, as the authors point out, these are available from the EPA anyway. However, the book must therefore be used in conjunction with an EPA approved method, or other source material to make accurate identification.

The authors have used a Hewlett-Packard 5992A "bench-top" spectrometer to obtain the spectra (one of the makes of instrument commonly used for EPA-style analyses). Quadrupoles can produce very variable spectra, and the authors point out this danger. Comparison of the spectra given in this compilation with other references, such as the Eight Peak Index or the EPA - NIH Mass Spectral Data Base, show most spectra to be similar, but occasionally relative abundances are very different. One way to standardise instruments is the use of decafluorotriphenylphosphine (DFTP) as a reference compound, and tuning the instrument to give an acceptable spectrum. The authors did this, so I feel it a great pity that the spectrum of DFTP was not included with those of the internal standards. This would give greater confidence for those spectra which differ from the spectra for the compound given in the two other major compilations.

Perhaps more importantly, identification of a compound should not be made on the basis of its spectrum alone. Indeed, many spectra in this book are virtually identical [the isomeric dichlorobenzenes; or dibenz(*a*)pyrene, benzo(*k*)fluoroanthene and benzo(*b*)fluoranthene]. Normally, identification of such specific components is made not only on the basis of their mass spectra but also on their relative retention time on gas-chromatographic analysis. This point is not made in the book, and is perhaps its major drawback if it is used for identification of specific pollutants. For a little extra effort the requisite gas-chromatographic traces could have been shown, and a retention-time index given for each pollutant. Also, when dealing with weak signals, and perhaps a high background, the relative abundances of ions can vary markedly. This point is not made, but when dealing with such a spectrum relative retention data are crucial to accurate identification.

However, the book does provide a single point source of reference spectra for the 114 priority pollutants. It does this very well and as such is a useful compendium for those who carry out occasional EPA-style analyses without the benefit of special software. However, the authors also wish the book to be used for the identification of specific pollutants in effluents, and the book cannot be used for this purpose as it stands at present, without the use of additional works.

Most instruments for EPA-style analyses have spectral libraries within the data system and spectra can be compared on line. I am not sure this book is needed at all, especially as the book is relatively expensive (in the UK anyway). N. J. HASKINS

WILSON AND WILSON'S COMPREHENSIVE ANALYTICAL CHEMISTRY. Volume XII. THERMAL ANALYSIS. Part A. SIMULTANEOUS THERMOANALYTICAL EXAMINATIONS BY MEANS OF THE DERIVATOGRAPH. By J. PAULIK and F. PAULIK. Pp. xviii + 277. Elsevier. 1981. Price $83; Dfl170. ISBN 0 444 41949 7.

This volume traces the first practical applications of simultaneous thermogravimetry and derivative thermogravimetry, by means of derivatography, some 25 years ago, to the more sophisticated "derivatographs" currently capable of recording, simultaneously, the curves attributable to temperature (T), thermodilatometry (TD), derivative thermodilatometry (DTD), thermogravimetry (TG), derivative thermogravimetry (DTG) and differential thermal analysis (DTA) or temperature (T), thermo-gas titrimetry (TGT), derivative thermo-gas titrimetry (DTGT), thermogravimetry (TG), derivative thermogravimetry (DTG) and differential thermal analysis (DTA). The term "derivatography," coined by the late Prof. Dr. Laszlo Erdey and the present authors, Drs. Jeno and Ferencz Paulik, is a term that is not recommended by the Nomenclature Committee of the International Confederation for Thermal Analysis (ICTA), and it is gratifying that, in the Preface, the authors acknowledge this fact and accordingly avoid using this term in the text. It is unfortunate, however, that the "Paulik Homers" nod occasionally by using the term "thermogram," instead of the ICTA Nomenclature Committee's recommendations of "TG curve," "DTA curve" or, more generally, "thermal analysis curve."

The monograph describes, in detail, the applications of "derivatography" to very many fields and it is unquestionably the most comprehensive book on the subject, to date, even though the authors state that "the collection of references is not complete." From a perusal of these references it is immediately apparent that most of the 1 268 cited emanate from the Eastern Bloc countries and it is a matter of some regret that, in the present reviewer's opinion, the "derivatograph" is not more readily available to the Western World.

The main criticism of this book is that it is not critical and reference to work other than "derivatography" is not cited. For example, when discussing the thermal decomposition of manganese, cobalt and nickel oxalates (p. 118), the phrase "according to one opinion" appears with no reference

to this "opinion." Further, when discussing the decomposition of catecholgermanic acid, the phrase "literature data are contradictory" is not supported by any references.

Errors are few and minor. In addition, the occasional odd phraseology can be forgiven from authors whose first language is not English. Nevertheless, for those thermal analysts who are interested in simultaneous techniques, the volume under review must be considered essential reading.

C. J. KEATTCH

PATTERN RECOGNITION IN CHEMISTRY. By KURT VARMUZA. *Lecture Notes in Chemistry, Volume 21*. Pp. xii + 217. Springer-Verlag. 1980. Price DM35.60; $21 (softback). ISBN 3 540 10273 6.

By chance, and good fortune, Prout's Bridgewater Treatise came to hand at the same time as the above, to provide an interesting contrast in style and a reminder that man has sought for patterns in nature for many years. However, whereas in the early 19th Century the skill lay in making generalisations of cosmic significance from a few facts, the modern scientist is faced with the problem of revealing the existence of more modest associations within a vast amount of data. Whilst the modern scientist might refrain from claiming that chemistry proves the existence of the Almighty, he can point out that pattern recognition methods have led to identification by X-ray spectra of specified elements in mixtures, assisted in the interpretation of mass spectra, classified unknown archaeological obsidian artefacts by place of origin and recognised counterfeit whisky. In all of these the original analytical information has been transformed mathematically and arranged according to some suitable program by a computer.

In this book, Dr. Varmuza critically reviews the many methods that have been experimented with. Those familiar with the concepts will find the survey most valuable. Each method is clearly outlined, numerous references are given, pitfalls and weaknesses are pointed out. There follows a comprehensive review of the application of pattern recognition methods to chemistry.

One of the weaknesses of a general method is that it may be difficult to apply to a specific example, hence the wide variety of methods available. Another is that a mountain of computation may produce a very predictable result (did it need a computer to detect counterfeit whisky?) or an absurd one (the case of successful classification of drugs by the number of characters in their names is cited). It is a strength of this book that the power of the method and its limitations are made evident. The incisive mind essential to the early workers is just as necessary in the era of the computer.

It is a state of the art subject, and it justifies a fulsome review such as this. The series, perhaps, dictates the style, with brevity being such a cardinal virtue that it is at times hard reading (quite the reverse from Prout). However, one warms to an author typing his manuscript in his second language on a mass spectrometric data system. The result is clear, the diagrams are good and the referencing excellent. In short, this can be strongly recommended as an advanced review of pattern recognition methods and their application to chemistry.

D. BETTERIDGE

Indirect Amplification Method for Determining Peroxydisulphate by Alternating-current Polarography

Short Paper

Keywords: *Peroxydisulphate determination*; *amplification*; *polarography*

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Analyst, 1981, **106**, 1217–1221.

Titrimetric Micro-determination of Peroxydisulphate by Amplification Reactions

Short Paper

Keywords: *Peroxydisulphate determination*; *amplification*; *iodimetry*

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Analyst, 1981, **106**, 1221–1224.

Indirect Spectrophotometric Determination of Iodide in Table Salts and Pharmaceutical Products

Short Paper

Keywords: *Iodide determination*; 3-(2′-*thiazolylazo*)-2,6-*diaminotoluene reagent*; *palladium complex*; *spectrophotometry*

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Analyst, 1981, **106**, 1224–1227.

Interference Due to Crystal Formation in the Spectrophotometric Determination of Iron(II) Using 2,4,6-Tri(2′-pyridyl)-1,3,5-triazine

Short Paper

Keywords: *Iron(II) determination*; *spectrophotometry*; 2,4,6-*tri*(2′-*pyridyl*)-1,3,5-*triazine*

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Analyst, 1981, **106**, 1227–1229.

Spectrophotometric Determination of Trace Amounts of Iron(III) with 2-(5-Chloro-2-pyridylazo)-5-diethylaminophenol

Short Paper

Keywords: *Iron determination*; *spectrophotometry*; 2-(5-*chloro*-2-*pyridylazo*)-5-*diethylaminophenol*

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Analyst, 1981, **106**, 1229–1233.

Spectrophotometric Determination of Micro-amounts of Chromate in the Presence of Iron(III), Chromium(III) and Other Ions

Short Paper

Keywords: *Chromium(VI) determination*; *spectrophotometry*; *iron(II)-FerroZine complex*

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Analyst, 1981, **106**, 1234–1237.

Dithiooxamide as a Reagent for the Detection and Spectrophotometric Determination of Chloral Hydrate in Alcoholic Beverages

Short Paper

Keywords: *Chloral hydrate determination*; *dithiooxamide*; *spectrophotometry*; *alcoholic beverages*

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Analyst, 1981, **106**, 1237–1239.

Sample Re-radiation Effects in the Quantitative Analysis of Crystalline Silica in Foundry Samples by Infrared Spectrophotometry

Communication

Keywords: *Crystalline silica*; *quartz*; *cristobalite*; *tridymite*; *infrared spectrophotometry*

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Analyst, 1981, **106**, 1240–1242.

A Toluidine Blue Stain Mountant for the Microscopy of Comminuted Meat Products

Communication

Keywords: *Microscopy*; *single-stage staining*; *meat tissues*; *wheat gluten*; *soya protein determination*

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Analyst, **1981**, **106**, 1242–1243.

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