The ANALYST

The Journal of the Society for Analytical Chemistry

A monthly International Publication
dealing with all branches of
Analytical Chemistry

Published by the
SOCIETY FOR ANALYTICAL CHEMISTRY

Volume 99

No. 1179, Pages 313-384 **June, 1974**

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THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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Published by The Society for Analytical Chemistry
Distributed by The Chemical Society, Publications Sales Office,
Blackhorse Road, Letchworth, Herts., SG6 1HN

Volume 99, No. 1179

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Summaries of Papers in this Issue

A Direct Gas-chromatographic Method for the Determination of Basic Nitrogenous Drugs in Pharmaceutical Preparations

A gas-chromatographic method has been developed for the direct assay of basic nitrogenous drugs, present as salts, in a range of pharmaceutical preparations. A glass column was packed with 3 per cent. silicone OV-17 on Gas-Chrom Q, 80 to 100 mesh, and maintained isothermally at temperatures within the range 200 to 270 °C, as appropriate for the drug to be determined. Calibration data are given for eighteen drugs, and the gas-chromatographic procedures have been subjected to a statistical evaluation. Five determinations performed on each of eight standard pharmaceutical preparations gave coefficients of variation of less than 2·2 per cent. The subsequent application of the method, based upon duplicate determinations, to a larger series of standard pharmaceutical preparations gave recoveries within the range 96 to 104 per cent. of the known concentration of the test drug. The procedure can now be used as part of a routine quality control specification in place of a variety of non-specific classical methods.

N. D. GREENWOOD and I. W. GUPPY

Regional Quality Control Laboratory, Pharmacy Department, Leeds General Infirmary, Great George Street, Leeds, LS1 3EX.

Analyst, 1974, **99**, 313–325.

A Method for the Determination of Volatile Fatty Acids in the Blood Plasma of Ruminant Animals

An improved method for the determination of volatile fatty acids in the blood plasma of ruminant animals is described. The acids are extracted as their sodium salts with isopropyl alcohol and then dissolved in 9 + 1 diethyl ether - formic acid for determination by gas - liquid chromatography.

J. W. GARDNER and G. E. THOMPSON

Department of Physiology, The Hannah Research Institute, Ayr, KA6 5HL, Scotland.

Analyst, 1974, **99**, 326–329.

Quantitative Determination of the Enantiomeric Purity of Synthetic Pyrethroids Part II. *S*-Bioallethrin

S-Bioallethrin consists primarily of (+)-allethronyl-(+)-*trans*-chrysanthemate, but technical samples contain small amounts of other allethrin isomers. The ratio of diastereoisomers can be measured directly from the nuclear magnetic resonance spectrum after using a europium shift reagent, when many of the resonances split into two distinct diastereoisomer signals. If the enantiomeric purity of the chrysanthemate moiety is determined independently, then the absolute enantiomeric purity of the allethrin sample can be calculated. Standard deviations for the measurement of laboratory or technical samples were between 0·3 and 0·7 per cent.

The accuracy of the method was verified by independently determining the enantiomer ratios of various allethrolone samples by gas chromatography of their diastereoisomeric (−)-α-methoxy-α-trifluoromethylphenylacetic esters, esterifying with natural (+)-*trans*-chrysanthemic acid and redetermining the enantiomeric purity by nuclear magnetic resonance. Good agreement was achieved between the two determinations.

The nuclear magnetic resonance method should also be suitable for measuring the diastereoisomer ratio of *cis*-allethrins.

F. E. RICKETT and P. B. HENRY

Wellcome Research Laboratories (Berkhamsted), Berkhamsted Hill, Berkhamsted, Hertfordshire.

Analyst, 1974, **99**, 330–337.

The Detection and Determination of Polynuclear Aromatic Hydrocarbons by Luminescence Spectrometry Utilising the Shpol'skii Effect at 77 K

The luminescence emission spectra of twenty-three polynuclear aromatic hydrocarbons (PAH) have been examined in n-alkane solvents at 77 K. The Shpol'skii effect, in which narrow-band (quasi-linear) emission spectra are obtained under these conditions when a monochromator of adequate resolving power is used, is shown to be readily observed for twelve of the compounds examined in these solvents. Quasi-linear emission spectra have also been obtained in tetrahydrofuran for some of the PAH compounds examined. These emission spectra provide for unambiguous qualitative identification of PAH compounds at trace concentrations in solution; this effect is demonstrated by identification of the compounds present in an eight-component mixture of PAH compounds.

Measurement of the low-temperature quasi-linear luminescence intensity can be applied quantitatively to the determination of these compounds provided that a standard additions procedure is employed in conjunction with the use of an internal standard to ensure sufficient accuracy and precision.

G. F. KIRKBRIGHT and C. G. de LIMA

Chemistry Department, Imperial College, London, S.W.7.

Analyst, 1974, **99**, 338–354.

Application of the Spectrophotometric Determination of Nickel and Cobalt in Mixtures With Bipyridylglyoxal Dithiosemicarbazone to the Analysis of Catalysts

Bipyridylglyoxal dithiosemicarbazone forms a complex with nickel(II) at pH 5·2, which can be extracted into chloroform ($\lambda_{max.} = 410$ nm). A similar complex is obtained with cobalt(II), but is not extractable in this solvent, thus allowing nickel and cobalt to be determined in mixtures. Two procedures are proposed for the accurate analysis of such mixtures in which 1 p.p.m. of one of the ions can be determined accurately in the presence of as much as 5 p.p.m. of the other. One of the procedures has been applied to the determination of nickel and cobalt in industrial catalysts and the results obtained have been compared with those obtained by atomic-absorption spectrophotometry. Satisfactory results were obtained.

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Department of Analytical Chemistry, University of Seville, Seville, Spain.

Analyst, 1974, **99**, 355–359.

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A Direct Gas-chromatographic Method for the Determination of Basic Nitrogenous Drugs in Pharmaceutical Preparations

BY N. D. GREENWOOD AND I. W. GUPPY

(Regional Quality Control Laboratory, Pharmacy Department, Leeds General Infirmary, Great George Street, Leeds, LS1 3EX)

A gas-chromatographic method has been developed for the direct assay of basic nitrogenous drugs, present as salts, in a range of pharmaceutical preparations. A glass column was packed with 3 per cent. silicone OV-17 on Gas-Chrom Q, 80 to 100 mesh, and maintained isothermally at temperatures within the range 200 to 270 °C, as appropriate for the drug to be determined. Calibration data are given for eighteen drugs, and the gas-chromatographic procedures have been subjected to a statistical evaluation. Five determinations performed on each of eight standard pharmaceutical preparations gave coefficients of variation of less than 2·2 per cent. The subsequent application of the method, based upon duplicate determinations, to a larger series of standard pharmaceutical preparations gave recoveries within the range 96 to 104 per cent. of the known concentration of the test drug. The procedure can now be used as part of a routine quality control specification in place of a variety of non-specific classical methods.

THE assay of basic nitrogenous drugs confronts the pharmaceutical analyst with a wide range of chemical and physico-chemical methods. Such methods include non-aqueous titration,[1–5] total nitrogen determination[2,6] and ultraviolet spectrophotometry,[2,7] all of which are open to the criticism of being non-specific towards a given drug, although they are included in several current pharmacopoeias.[5,8–13] The tetraphenylboron precipitation method of Johnson and King[14] and Bonnard,[15] has been applied to the assay of alkaloids in eye drops[16,17] and is currently specified in the B.P.C.[18] Other non-specific titrimetric methods[2,19–21] can also be used in the assay of basic nitrogenous drugs, and again several such methods are specified in the various pharmacopoeias.[9,22]

Gas - liquid chromatography permits a standard procedure to be applied to the majority of preparations, which is far more specific than alternative "classical" methods. The examination by gas - liquid chromatography of many basic nitrogenous drug substances (in an organic solvent) has been reported in the literature,[23–37] and it has become a standard method for their determination in biological materials.[37–45] These drugs are conventionally chromatographed as the free bases after extraction (if necessary) from alkaline solution into a suitable organic solvent.[23,26,29,32,46–50]

Gas chromatography is by now a well established technique in pharmaceutical analysis[23,26,47–64] and its applications within a hospital pharmaceutical laboratory have been discussed.[62–64] A direct approach will often be indicated for the examination of basic nitrogenous drugs as they are normally present in pharmaceutical preparations as the stable salt of a mineral acid in aqueous solution.

The direct injection of the salts of basic nitrogenous drugs has been reported[23,26,29,35,46,65–70] (see also Greenwood, N. D., unpublished work) and would be expected to result in the on-column liberation of the corresponding free bases, either by thermal dissociation,[35,66,68] or on account of the natural basicity of the support.[23] Many reports regarding injection as the salts are of a qualitative nature, although some quantitative results have been reported, notably by Koehler and Hefferren,[23] who advocated a direct method for the determination of a range of local anaesthetics in pharmaceutical preparations, and Rader and Aranda,[34] who examined a wide range of drugs. Umbreit, Nygren and Testa[71] evaluated methods for the direct determination of trace amounts of the salts of several aliphatic amines in water samples.

The typical adsorption problems that are associated with basic nitrogenous compounds in general[29,71–80] may be encountered with these drugs, leading to the phenomena of ghosting[63,75,81] and tailing.[82–84] These effects can be counteracted by various means, including silanisation of the support,[85–87] modification of the support with alkali,[26,29,47,56,65,66,71–74,84,88,89] the use of an alkaline pre-column,[65,71,90–92] the coating of a low loading of stationary phase on to glass micro-beads[23,26,93–95] or the preparation of volatile derivatives.[30,71,96] This last approach was rejected, because it could involve complex and time-consuming procedures.

The use of a very inert support such as Gas-Chrom Q (Applied Science Laboratories) would be expected to minimise the adsorption effects, and Alber[67] has advocated the use of 3·0 per cent. silicone OV-17 on this support as an "all purpose gas - liquid chromatographic column for pharmaceuticals." In a series of chromatograms, the resolution of a comprehensive range of drugs was illustrated, and the examples included a number of basic nitrogenous drugs, some of which were injected directly as a salt. Good peak symmetry was maintained even for those drugs with comparatively long retention times, but no quantitative results were reported, nor was the significance of this phenomenon discussed.

A variety of stationary phases can be used in the analysis by gas - liquid chromatography of basic nitrogenous compounds,[23,25,28,95] the most commonly reported being silicones.[23–29,33,35,54,57,76,91] The increased thermal stability of the OV silicones over the established silicones such as SE-30 is obviously advantageous when dealing with comparatively involatile drugs; this stability is of particular significance when drugs such as codeine, morphine, papaverine and quinidine, which require high column temperatures in order to obtain symmetrical peaks within a reasonable retention time, are to be chromatographed on the same column as less complex drugs, such as lignocaine, pethidine and procaine. Even so, problems may be encountered with those drugs, such as ephedrine and fenfluramine, which require comparatively low column temperatures.

Thus, columns such as that described by Alber[67] and others[97,98] would appear to present an ideal choice, as they combine an OV silicone with a very inert support. That they are suitable was confirmed by a preliminary evaluation, in which almost perfect peak symmetry was observed from a wide range of drugs. A typical chromatogram is shown in Fig. 1, which illustrates the resolution of several local anaesthetics covering a range of structures.

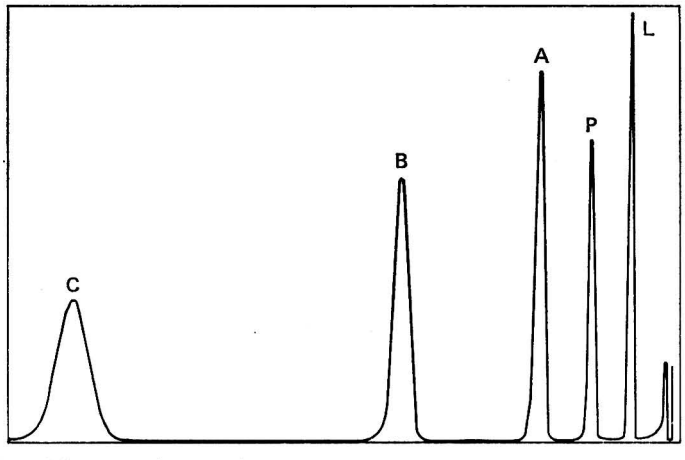

Fig. 1. Mixture of local anaesthetics injected as the salts; A, amethocaine hydrochloride; B, butacaine sulphate; C, cinchocaine hydrochloride; L, lignocaine hydrochloride monohydrate; and P, procaine hydrochloride. Column temperature, 235 °C

GLOSSARY OF TERMS—

The following terminology is used throughout this paper.

Standard solution—A solution containing a known concentration of the drug under test in aqueous solution (normally 1·0 per cent. *m*/*V*).

Internal marker solution—A solution containing a known concentration of the compound to be used as the internal marker in a given assay (normally 1·0 per cent. *m/V*).

Reference solution—A solution containing an aliquot of the standard solution together with the fixed amount of the internal marker and diluted with water to the required volume (normally 25·0 ml).

Dilution—The preliminary dilution of the sample (if necessary) prior to the preparation of the test solution.

Test solution—A solution containing an aliquot of the sample under test, together with the fixed amount of the internal marker for a given assay, and diluted to the required volume with water (normally 25·0 ml).

Standard pharmaceutical preparation—A preparation prepared according to the formula usually used within this department, or as specified in a pharmacopoeia, so as to contain a known concentration of a test drug.

Experimental

Materials—

All of the drugs were to B.P. or B.P.C. specification as appropriate, with the exceptions of procaine hydrochloride and papaverine hydrochloride, which were of laboratory-reagent grade. The other constituents, including antibacterials, antioxidants and stabilisers, were of grades suitable for incorporation into pharmaceutical preparations.

Reagents—

For calibration purposes a standard 1·0 per cent. *m/V* aqueous solution of each drug was prepared and stored in a sealed amber-glass bottle for subsequent stability studies. The formulated products were prepared in accordance with the specification used in the manufacturing units within this hospital, normally B.P. or B.P.C., as set down in the relevant tables.

Gas chromatography—

A Pye Series 104 gas chromatograph, fitted with dual flame-ionisation detectors, was used in conjunction with a 10 mV full-scale deflection potentiometric recorder and an electronic integrator. The chromatograph included a wide-range amplifier module, thus enabling the integrator to be used to full advantage.

A glass column, 1 m long by 4 mm internal diameter, was packed with 3 per cent. silicone OV-17 on Gas-Chrom Q, 80 to 100 mesh[67] (Phase Separations Ltd.), which had been conditioned at 270 °C overnight. Argon was used as carrier gas at a flow-rate of 50 ml min^{-1}. The detectors were maintained at the same temperature as the column, and the flame gases were hydrogen at 50 ml min^{-1} and air at 600 ml min^{-1}.

Aliquots of each solution (of about 1 μl) were injected into a heated zone above the column packing, at a temperature about 50 °C higher than that of the column (corresponding to setting number 2 on the heater control). The column temperatures appropriate to each drug investigated in this study are listed in Table I.

Only one column position was used at any given time; no balancing of column bleed rates was attempted, as a steady base-line was obtained at the attenuation settings that were employed during this work.

Calibration procedures—

Aliquots (1, 2, 3, 5, 7 and 10 ml) of the standard solution of the drug under test were pipetted into a series of 25-ml calibrated flasks and either 3·0 or 5·0 ml of the internal marker solution (equivalent to 30 or 50 mg) were added, the amount depending upon the detector response to the two compounds involved. It was necessary to substitute a 15-ml aliquot of the standard quinidine sulphate solution in place of the 1-ml aliquot. Details of the internal marker applicable to a given drug are listed in Table I. Similar calibrations were prepared for certain drugs which may also be present at concentrations below those covered by these graphs, namely atropine sulphate and hyoscine hydrobromide, by using solutions of the test drug and internal marker at concentrations of 0·1 per cent.

TABLE I

SUMMARY OF INTERNAL MARKERS, GAS - LIQUID CHROMATOGRAPHIC CONDITIONS AND CALIBRATION DATA

Volume of test solution = 25 ml

Test drug	Internal marker*		Gas - liquid chromatographic column temperature/°C	Linear range of the calibration graph/mg
	Identity	Amount/mg		
Amethocaine hydrochloride	B	50	240	20 to 100
Atropine sulphate	B	50	245	20 to 100
Butacaine sulphate	Ci	50	255	10 to 100
Cinchocaine hydrochloride	B	30	255	20 to 100
Cocaine hydrochloride	D	30	235	10 to 100
Codeine phosphate	Ci	50	255	10 to 100
Eserine sulphate	Cm	30	220	10 to 50
Homatropine hydrobromide	D	30	235	10 to 100
Hyoscine hydrobromide	Ci	50	255	20 to 100
Lignocaine hydrochloride monohydrate	Cm	50	205	10 to 100
Morphine sulphate	Ci	50	255	20 to 100
Oxybuprocaine hydrochloride	P	30	235	10 to 100
Papaverine hydrochloride	Ci	50	270	20 to 100
Papaverine sulphate	Ci	50	270	20 to 100
Pethidine hydrochloride	Cm	50	210	10 to 100
Pilocarpine hydrochloride	B	50	245	10 to 100
Procaine hydrochloride	D	30	215	20 to 100
Quinidine sulphate	Ci	50	270	20 to 150

* Added as a 1·0 per cent. *m*/*V* solution; 1 ml = 10 mg. Internal markers: B, butacaine sulphate; Ci, cinchocaine hydrochloride; Cm, chlorpheniramine maleate; D, diphenhydramine hydrochloride; and P, procaine hydrochloride.

The peak area ratio of the test drug to internal marker was then calculated. The method was calibrated in duplicate for each drug by plotting a graph of the peak area ratio *versus* its concentration, thus establishing the linear range applicable to a given drug (see Table I). Subsequently, only two reference solutions were injected alongside each test solution in order to define the slope of the line.[99]

Certain drugs, including atropine sulphate, eserine sulphate and hyoscine hydrobromide, decomposed on-column,[35,53] thus giving rise to two or more major peaks, as illustrated in Fig. 2. Nevertheless, accurate calibration data could be obtained by using the summed areas of the peaks, although this on-column decomposition detracts from the ability of the

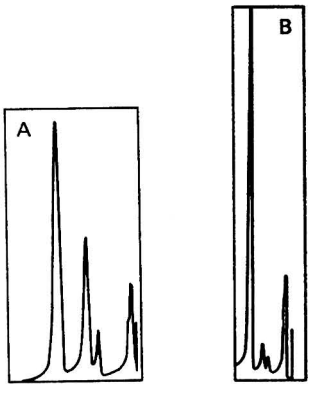

Fig. 2. Typical chromatograms from drugs that exhibit on-column decomposition. A, atropine sulphate (column temperature, 245 °C); and B, eserine sulphate (column temperature, 220 °C)

method to detect breakdown products within the actual preparations. This phenomenon became exaggerated with atropine methonitrate, which gave several peaks, thus preventing accurate measurement of the peak areas.

The analysis by gas - liquid chromatography of tropane alkaloids in general, has been reviewed by Achari and Newcombe[100] and the determination of atropine and hyoscine in pharmaceutical preparations is well documented.[101]

Ephedrine hydrochloride could not be satisfactorily chromatographed on this column because at lower temperatures excessive tailing was observed and, if the temperature was elevated, it was eluted on the tail of the solvent peak. Data relating to the gas - liquid chromatography of this drug are, however, particularly well documented in the literature.[34,39,46,102–104]

STATISTICAL EVALUATIONS—

A series of reference solutions (normally ten) containing the drug under test (50 mg), together with the appropriate internal marker, was prepared for each of the three drugs cinchocaine hydrochloride, lignocaine hydrochloride monohydrate and procaine hydrochloride.

Fifteen aliquots (each about 1 μl) of one solution in each series, together with certain other test solutions, were injected in order to ascertain the precision that can be expected from the gas - liquid chromatographic system. The peak area ratio arising from each injection, and hence the over-all standard deviation (σ), was calculated, σ being defined thus:

$$\sigma = \sqrt{\frac{N\,\Sigma X^2 - (\Sigma X)^2}{N\,(N-1)}}$$

where X represents the sum of N individual peak area ratios.

Two aliquots (of about 1 μl) of each solution were then injected and the average peak area ratio was calculated for each solution. Again, the standard deviation was calculated for the series of solutions in order to ascertain the over-all precision of the dilution and the gas - liquid chromatographic procedures. The calculations were performed on an Olivetti Programma 101 electronic desk computer.

GENERAL PROCEDURE—

An amount of the sample within the linear range of the calibration graph was added to a 25-ml calibrated flask. The internal marker was then added as a 1·0 per cent. m/V solution and the contents of the flask were diluted to the mark.

Two aliquots of a standard solution of the drug under test were also diluted in a similar manner, so as to contain concentrations close to that of the sample; one of these aliquots would normally correspond to the concentration expected from the sample. Full details of the sampling procedure are given in Table II.

TABLE II

SAMPLING INFORMATION REGARDING TYPICAL PHARMACEUTICAL PREPARATIONS

Test drug	Preparation and standard*	Concentration of the test drug, per cent. m/V	Aliquot to be taken for the assay/ml	
			Sample	Reference
Amethocaine hydrochloride ..	Eye drops, B.P.C.	1·00	5·0	7·0, 5·0
	Pastilles, L.G.I.	6·00†	‡	7·0, 5·0
	Topical solution, L.G.I.	2·00	3·0	7·0, 5·0
Atropine sulphate	Eye drops, B.P.C.	1·00	5·0	7·0, 5·0
		0·50	10·0	7·0, 5·0
		0·125	5·0§	7·0, 5·0§
	Injection, B.P.	0·06	10·0§	7·0, 5·0§
		0·04	15·0§	7·0, 5·0§
Butacaine sulphate	Eye drops, L.G.I.	0·50	10·0	7·0, 5·0
Cinchocaine hydrochloride ..	Jelly, L.G.I.	2·00†	¶	7·0, 5·0
Cocaine hydrochloride ..	Eye drops, B.P.C.	4·00	10·0 to 50·0 take 10·0	8·0, 5·0
		2·00	3·0	7·0, 5·0
(*plus* adrenaline, 1 + 999)	Eye drops, L.G.I.	5·00	5·0 to 50·0 take 10·0	7·0, 5·0

TABLE II—continued

Test drug	Preparation and standard*	Concentration of the test drug, per cent. *m/V*	Aliquot to be taken for the assay/ml: Sample	Aliquot to be taken for the assay/ml: Reference
Codeine phosphate	Injection, L.G.I.	6·00	5·0 to 50·0 take 10·0	7·0, 5·0
Homatropine hydrobromide ..	Eye drops, B.P.C.	2·00	3·0	7·0, 5·0
		1·00	5·0	7·0, 5·0
Hyoscine hydrobromide ..	Eye drops, L.G.I.	1·00	5·0	7·0, 5·0
	Eye drops, B.P.C.	0·50	10·0	7·0, 5·0
Lignocaine hydrochloride monohydrate	Injection, B.P.	5·00	5·0 to 50·0 take 10·0	7·0, 5·0
		2·00	3·0	7·0, 5·0
		1·50	5·0	8·0, 5·0
		1·00	5·0	7·0, 5·0
		0·50	10·0	7·0, 5·0
		0·125	15·0	3·0, 2·0
	Topical solution, L.G.I.	4·00	5·0 to 50·0 take 15·0	7·0, 5·0
Morphine sulphate	Injection, B.P.	1·50	3·0	7·0, 5·0
		1·00	5·0	7·0, 5·0
Oxybuprocaine hydrochloride (*plus* fluorescein sodium) ..	Eye drops, L.G.I.	0·40	10·0	5·0, 3·0
		0·30	15·0	5·0, 3·0
Papaverine hydrochloride ..	Solution, L.G.I.	2·50	10·0 to 50·0 take 10·0	7·0, 5·0
Papaverine sulphate	Isoprenaline spray co., B.P.C.	2·50	10·0 to 50·0 take 10·0	7·0, 5·0
Pethidine hydrochloride ..	Injection, B.P.	5·00	10·0 to 50·0 take 5·0	7·0, 5·0
		1·00	5·0	7·0, 5·0
Pilocarpine hydrochloride ..	Eye drops, B.P.C.	4·00	5·0 to 50·0 take 15·0	7·0, 5·0
		3·00	10·0 to 50·0 take 10·0	7·0, 5·0
		2·00	3·0	7·0, 5·0
		1·00	5·0	7·0, 5·0
	Injection, L.G.I.	2·00	3·0	7·0, 5·0
Procaine hydrochloride ..	Injection, L.G.I.	2·00	3·0	7·0, 5·0
		1·00	5·0	7·0, 5·0
		0·50	10·0	7·0, 5·0
		0·125	20·0	3·0, 2·0
(*plus* adrenaline, 1 + 49 999)	Injection, B.P.	2·00	3·0	7·0, 5·0
Quinidine sulphate	Injection, L.G.I.	6·00	10·0 to 50·0 take 10·0	15·0, 10·0
Compound preparations—				
Cocaine hydrochloride *plus* homatropine hydrobromide	Eye drops, B.P.C.	4·00	10·0 to 50·0 take 10·0	8·0, 5·0**
		2·00		5·0, 3·0**
Cocaine hydrochloride *plus* homatropine hydrobromide	Eye drops, B.P.C.	2·00	3·0	7·0, 5·0**
		2·00		7·0, 5·0**

* Where a preparation is not the subject of a monograph in the 1968 editions of the British Pharmacopoeia (B.P.) or British Pharmaceutical Codex (B.P.C.), the formula prepared within this hospital (L.G.I.) is given.

† Solid and semi-solid preparations are expressed as a percentage *m/m*.

‡ The preliminary dilution for amethocaine hydrochloride pastilles is prepared by dissolving about 5 g in warm distilled water (about 40 ml) in a 100-ml calibrated flask. The solution is then cooled and diluted to the mark, and 15·0 ml are taken for the assay.

§ The concentrations of the internal marker and standard reference solutions are 0·10 per cent. *m/V*.

¶ The test solution for cinchocaine hydrochloride jelly is prepared by transferring about 2·5 g of the sample to a 25-ml calibrated flask; the internal marker is then added, followed by distilled water to a volume of about 20 ml. The jelly is then dispersed throughout the solution, which is diluted to the mark, after the frothing has been allowed to subside.

** The reference solutions for the simultaneous determination of two drugs will contain both of the test drugs *plus* the internal marker. For example:

cocaine hydrochloride	80 mg
homatropine hydrobromide ..	50 mg
diphenhydramine hydrochloride ..	30 mg
distilled water	to 25 ml

Two aliquots (of about 1 μl) of each of the three solutions were then injected in a sequence that would minimise the effects of any short-term instability in the chromatographic system. The results were calculated from the following equation[99] to the full capacity of the laboratory calculator (floating decimal point) and subsequently rounded to the required number of decimal places (two or three).

$$C_S = \left[\frac{R_S\,(C_1 - C_2)}{R_1 - R_2} + C_1 - \frac{R_1\,(C_1 - C_2)}{R_1 - R_2}\right] \times \frac{V}{A} \times D$$

where C_S is the concentration of the test drug in the sample, R_S, the peak area ratio resulting from the test solution, C_1 and C_2 are the concentrations of the test drug in the two reference solutions, giving peak area ratios R_1 and R_2, respectively, V is the volume of the test solution (normally 25·0 ml), A, the volume of the aliquot of the sample taken for the assay and D, the dilution of the sample (if any). C_S, C_1 and C_2 can be in per cent., m/V or m/m as appropriate. A, V and D are all normally expressed in millilitres, except for solid or semi-solid preparations, when A is expressed in grams.

EVALUATION OF THE METHOD—

A series of eight typical pharmaceutical preparations, containing known concentrations of amethocaine hydrochloride, lignocaine hydrochloride monohydrate, oxybuprocaine hydrochloride or procaine hydrochloride, was prepared, and five replicate determinations were performed on each preparation. The results from each analysis were then subjected to a statistical evaluation in order to ascertain the reliability of the method as a whole.

The evaluation of the method was extended according to a procedure described previously.[99] Duplicate assays were performed on a number of typical pharmaceutical preparations containing known concentrations of the test drug. They were not sterilised, as this procedure could cause some decomposition. All of the assays were performed at room temperature with the exception of the eye drops containing oxybuprocaine hydrochloride (0·3 per cent. m/V) *plus* fluorescein sodium (0·125 per cent. m/V), which were sampled at 37 °C in order to avoid precipitation.[105,106]

Certain more complex formulations, notably oral medications, cannot be assayed by this direct method because they give rise to a large, tailing solvent peak. This effect is typified by Codeine Linctus B.P.C.,[107] which contains 0·3 per cent. m/V of codeine phosphate. This drug is eluted on the solvent peak but accurate quantitative results could not be obtained by the measurement of peak areas or by triangulation of the peak height measurements. A typical chromatogram is illustrated in Fig. 3. No results relating to such preparations are reported in this paper, but methods based on an extraction of the free base are documented in the literature.[27,29,33,104]

SIMULTANEOUS DETERMINATION OF COMPOUND PREPARATIONS—

Certain preparations include two (or more) basic nitrogenous drugs, and typical formulations were evaluated according to the standard procedure, by using reference solutions containing both compounds in admixture. As part of the calibration procedure, it was established that no interaction occurred between the two drugs by the examination of two additional series of solutions containing various amounts of one drug in the presence of a fixed amount of the other.

The application of the method to compound preparations is dependent upon two principal factors, namely the concentration of the two drugs, and their resolution by the gas - liquid chromatographic system. The selection of a compound that is eluted between the two test drugs represents the optimum choice as the internal marker. Although cocaine hydrochloride and homatropine hydrochloride, which may be present together in certain eye drop formulations,[108] can be resolved by the gas - liquid chromatographic system at a temperature of 235 °C (Fig. 4), any significant reduction in the temperature results in excessive tailing of both peaks, thus precluding the use of an internal marker that is eluted with an intermediate retention time. Three compounds were evaluated in a preliminary series of experiments, but diphenhydramine, which is eluted before both drugs, was selected as the internal marker for their simultaneous assay as it gave the most reproducible results. The simultaneous determination of these two drugs as the free bases has been suggested previously.[109]

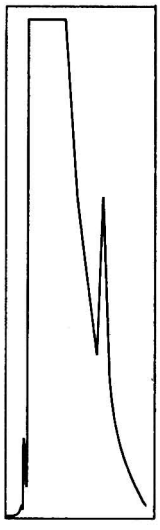

Fig. 3. Typical chromatogram following the direct injection of Codeine Linctus B.P.C. (diluted 1 in 5 with water). Column temperature, 255 °C

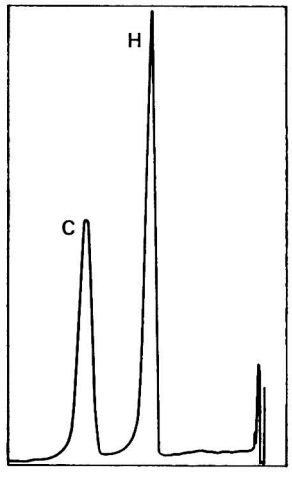

Fig. 4. Chromatogram obtained following the injection of cocaine hydrochloride (C) *plus* homatropine hydrobromide (H). Column temperature, 235 °C

STABILITY OF THE REFERENCE SOLUTIONS—

The use of freshly prepared standard solutions on each occasion is time consuming, especially in a laboratory that handles a large number of samples. In order to ascertain the feasibility of a monthly preparation regimen, a series of standard 1·0 per cent. *m/V* aqueous solutions of certain drugs (see Table VI) were stored in sealed, amber-glass bottles at room temperature for a period of at least 4 weeks. No antibacterial agents or chemical preservatives were added.

Each stored solution was then examined by gas - liquid chromatography alongside a freshly prepared standard solution of the same drug. A 5·0-ml aliquot of each solution was diluted to 25·0 ml, together with the appropriate internal marker (see Table I), and two aliquots (of about 1 μl) of each dilution were injected into the chromatograph. The average peak area ratio was calculated for each solution, and the recovery of the test drug in the stored solution was calculated from the equation—

$$\text{Recovery, per cent.} = \frac{\text{Mean peak area ratio for stored solution}}{\text{Mean peak area ratio for fresh solution}} \times 100$$

RESULTS AND DISCUSSION

The chromatographic results confirm that excellent peak symmetry and good resolution of closely related structures can be expected, even from those drugs with comparatively long retention times (see Fig. 1). In general, peak tailing was absent, but certain drugs did exhibit a minimal degree of tailing, which did not adversely affect the accuracy or precision. Although the gas - liquid chromatographic column can be maintained at elevated temperatures (about 250 °C) for prolonged periods of time (1 to 2 months) it was found to be necessary to repack the initial portion of the column every 2 to 3 months. The entire column was repacked after 4 to 6 months, when indicated by a deterioration in peak symmetry and in the reproducibility of peak area ratios between duplicate injections of the same solution. Deterioration may be partly due to the effects of the acids that are liberated by the dissociation of the salts, although no adverse effects upon the flame-ionisation detector arising from this source[110] have been observed.

The calibration data are summarised in Table I, and the linear working range covers test solutions at least containing between 20 and 100 mg of the test drug, with the exception of quinidine sulphate. The calibration graphs prepared for atropine sulphate and hyoscine hydrobromide, based upon the 0·1 per cent. m/V solutions, both gave a linear range from 1 to 10 mg.

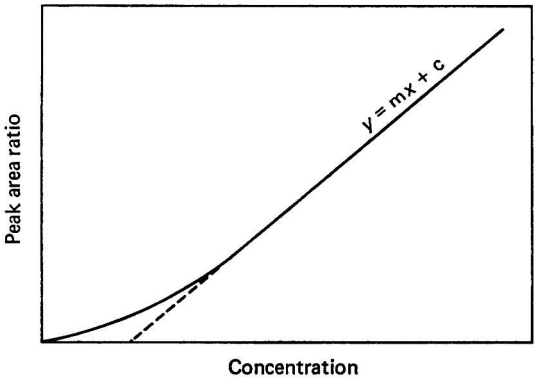

Fig. 5. Calibration graph typically obtained with involatile drugs

Most of the drugs investigated in this study gave rise to the typical calibration graph of involatile compounds[29,99,111–113] shown in Fig. 5, in which an initial non-linear portion gives way to a wider linear working range, which, if produced, intersects the y-axis at some point other than the origin. The significance of this type of calibration graph has been discussed previously[99] and gives rise to the necessity to prepare two reference solutions. Once the linear working range for a given drug has been established, it is not necessary to construct a full graph during the routine application of the method, but merely to define the slope of the line.

TABLE III

STATISTICAL EVALUATIONS OF THE GAS-CHROMATOGRAPHIC PROCEDURES

(a) *Replicate injections of one solution—*

Test drug*	Number of injections	Mean peak area ratio	Standard deviation	Coefficient of variation, per cent.
Atropine sulphate	10	1·078	0·054	5·01
Cinchocaine hydrochloride ..	15	1·741	0·029	1·67
Cocaine hydrochloride†	10	1·166	0·037	3·17
Homatropine hydrobromide† ..	10	0·984	0·018	1·83
Lignocaine hydrochloride monohydrate	15	1·172	0·009	0·77
Procaine hydrochloride	15	1·086	0·038	3·50

(b) *Duplicate injections of several solutions—*

Test drug*	Number of injections	Mean peak area ratio‡	Standard deviation§	Coefficient of variation, per cent.
Cinchocaine hydrochloride ..	8	1·745	0·013	0·74
Lignocaine hydrochloride monohydrate	10	1·180	0·022	1·86
Procaine hydrochloride	10	1·089	0·020	1·84

* Internal markers as specified in Table I.
† Determined simultaneously.
‡ Mean of the individual mean peak area ratios from each solution.
§ Applied to the mean peak area ratios from each solution.

The statistical evaluations of the gas - liquid chromatographic system and the sampling techniques are summarised in Table III. As might be expected, atropine sulphate, which decomposed on-column, gave a higher coefficient of variation than the other drugs not demonstrating this effect. The data resulting from the application of the full method to representative series of determinations, which are reported in Table IV, indicate that a high degree of accuracy and precision can be expected. The results from the two concentrations of amethocaine hydrochloride in eye drops confirm that the procedure will readily differentiate between preparations containing similar concentrations of the same drug. Coefficients of variation of about 2 per cent. or less were obtained from all these preparations.

TABLE IV

STATISTICAL EVALUATION OF THE METHOD AS APPLIED TO TYPICAL PHARMACEUTICAL PREPARATIONS

Test drug and preparation	Concentration of the test drug, per cent. *m*/*V* Added	Found*	Standard deviation†	Standard error of the mean‡	Coefficient of variation, per cent.
Amethocaine hydrochloride eye drops, B.P.C. 1968	0·95	0·95	0·012	0·005	1·26
Amethocaine hydrochloride eye drops, B.P.C. 1968	1·10	1·09	0·024	0·011	2·20
Lignocaine hydrochloride monohydrate injection, B.P. 1968	0·50	0·50	0·009	0·004	1·80
Lignocaine hydrochloride monohydrate injection, B.P. 1968	5·10	5·17	0·053	0·024	1·03
Lignocaine hydrochloride monohydrate injection, B.P. 1968 (*plus* adrenaline, 1 + 99 999)	2·00	1·96	0·026	0·012	1·33
Oxybuprocaine hydrochloride eye drops	0·41	0·40	0·005	0·002	1·25
Oxybuprocaine hydrochloride eye drops (*plus* fluorescein sodium, 0·125 per cent. *m*/*V*)	0·30	0·30	0·006	0·003	2·00
Procaine hydrochloride injection, U.S.P. (1970)	1·05	1·05	0·008	0·004	0·76

* Mean of five determinations.
† Defined in the text.
‡ Standard error of the mean is defined thus: S.E.M. $= \frac{\sigma}{\sqrt{N}}$.

A detailed comparison of the gas - liquid chromatographic procedure with alternative methods was not undertaken as part of the investigation, because chromatography is validated by the statistical data and the results obtained from its subsequent application to typical analyses, which are summarised in Table V.

Recoveries within the range from 96 to 104 per cent. of the amount of test drug known to be present were obtained during the application of the method to a wide range of drugs, including the simultaneous assay of cocaine hydrochloride and homatropine hydrobromide. These recoveries are similar to those reported previously by Rader and Aranda.[34]

Eye drops containing eserine sulphate, which decomposes on-column, gave variable recoveries, but a gas-chromatographic method of assay for this drug as the trimethylsilyl derivative has been described previously.[114] The preparations containing atropine and hyoscine yielded good quantitative results. Certain drugs among those which required a comparatively high column temperature, such as morphine sulphate and papaverine hydrochloride, did not yield reproducible recoveries within the set limits and consequently no results for them are reported in Table V. Similarly, papaverine and quinidine sulphates gave variable results, but the effect was not so marked. No explanation for this phenomenon is evident, but repeated assays on the standard preparations gave varied recoveries over an unacceptably wide range. The salts of papaverine gave over-all borderline results, and the method cannot be regarded as being satisfactory for this drug.

The stability studies on the standard reference solutions of certain drugs, which are reported in Table VI, confirm that, in general, the replacement of these solutions at monthly intervals is justified. It is axiomatic that the raw materials to be used in the preparation of these reference solutions should be subjected to assay by an appropriate method, in order to ascertain accurately their purity.

TABLE V

SUMMARY OF RESULTS FROM THE APPLICATION OF THE GAS - LIQUID CHROMATOGRAPHIC METHOD TO A SERIES OF TYPICAL PHARMACEUTICAL PREPARATIONS

Test drug	Preparation and standard*	Concentration of the test drug, per cent. *m/V*	Results by gas - liquid chromatography‡	
			Concentration, per cent. *m/V*	Average recovery, per cent.
Amethocaine hydrochloride	Pastilles, 1 g, L.G.I.	5·87†	5·78, 5·90	99·5
	Topical solution, L.G.I.	2·00	1·98, 1·98	99·0
Atropine sulphate	Eye drops, B.P.C.	1·00	1·02, 1·04	103·0
	Eye drops, B.P.C.	0·50	0·52, 0·52	104·0
	Eye drops, B.P.C.	0·125	0·121, 0·128	99·6
	Injection, B.P.	0·06	0·059, 0·064	102·5
Butacaine sulphate	Eye drops, L.G.I.	0·50	0·51, 0·51	102·0
Cinchocaine hydrochloride	Jelly, L.G.I.	2·00†	2·04, 2·06	102·5
Cocaine hydrochloride	Eye drops, B.P.C.	4·05	4·12, 4·06	101·0
(*plus* adrenaline, 1 + 999)	Eye drops, L.G.I.	5·00	4·89, 4·99	98·8
Codeine phosphate	Injection, L.G.I.	6·00	6·08, 6·00	100·7
Homatropine hydrobromide	Eye drops, B.P.C.	2·10	2·06, 2·09	98·8
	Eye drops, B.P.C.	1·05	1·04, 1·04	99·1
Hyoscine hydrobromide	Eye drops, L.G.I.	0·50	0·50, 0·51	101·0
Lignocaine hydrochloride monohydrate	Injection, B.P.	1·50	1·54, 1·53	102·3
	Topical solution, L.G.I.	4·00	4·04. 4·05	101·3
Papaverine sulphate	Isoprenaline spray co., B.P.C.	2·50	2·55, 2·58	102·6
Pethidine hydrochloride	Injection, B.P.	5·00	5·14, 5·05	101·9
Pilocarpine hydrochloride	Eye drops, B.P.C.	4·00	3·98, 3·86	98·0
	Eye drops, B.P.C.	3·00	2·96, 2·86	97·0
	Eye drops, B.P.C.	1·00	0·99, 1·02	100·5
	Injection, L.G.I.	2·00	1·96, 1·97	98·3
Procaine hydrochloride	Injection, L.G.I.	0·50	0·48, 0·48	96·0
	Injection, L.G.I.	0·125	0·124, 0·123	98·8
(*plus* adrenaline, 1 + 49 999) ..	Injection, B.P.	2·00	1·95	97·5
Quinidine sulphate	Injection, L.G.I.	6·07	6·24, 5·84	99·5
Compound preparations—				
Cocaine hydrochloride	Eye drops, B.P.C.	4·00	3·93, 3·82	96·9
(*plus* homatropine hydrobromide) ..		1·95	1·93, 1·87	97·4
Cocaine hydrochloride	Eye drops, B.P.C.	2·00	1·96, 1·96	98·0
(*plus* homatropine hydrobromide) ..		2·05	2·12, 2·08	102·4

*, † Defined in the footnotes to Table II.

‡ In general, the assays were performed in duplicate, the recoveries being based upon the average of the two determinations.

TABLE VI

SUMMARY OF RESULTS FROM THE STABILITY STUDIES ON THE STANDARD DRUG SOLUTIONS

Test drug	Length of time under storage/weeks	Recovery, as compared with a freshly prepared solution, per cent.
Atropine sulphate	8	99·4
Cinchocaine hydrochloride ..	6	100·4
Cocaine hydrochloride	8	99·5
Codeine phosphate	7	99·8
Homatropine hydrobromide ..	9	101·9
Hyoscine hydrobromide ..	5	98·9
Morphine sulphate	7	101·3
Papaverine hydrochloride ..	7	98·4
Pilocarpine hydrochloride ..	9	100·9
Quinidine sulphate	8	103·0

Conclusions

A statistical evaluation of the gas-chromatographic method has shown that satisfactory results can be obtained. This was confirmed during the subsequent application of the method to a series of typical pharmaceutical preparations that contain a number of basic nitrogenous drugs as salts.

The method has now been in routine use for over 18 months and has proved to be reliable and convenient.

The authors are grateful to all their colleagues who have assisted in various aspects of the study, particularly Mr. C. Hetherington and Dr. H. E. Nursten, for helpful discussions, and Miss E. Walker, for the preparation of the manuscript. We are indebted to the Department of Medicine, University of Leeds, for the use of the Olivetti computer.

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Received *September 14th*, 1973
Accepted *December 17th*, 1973

A Method for the Determination of Volatile Fatty Acids in the Blood Plasma of Ruminant Animals

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An improved method for the determination of volatile fatty acids in the blood plasma of ruminant animals is described. The acids are extracted as their sodium salts with isopropyl alcohol and then dissolved in 9 + 1 diethyl ether - formic acid for determination by gas - liquid chromatography.

THE accurate determination of volatile fatty acids in human or animal blood is difficult to achieve and has been attempted by a variety of methods.[1–9] Many of these methods have involved the use of steam distillation, but it has been shown that steam distillation of the small amounts involved here is an irreproducible process and that considerable losses may occur.[10] In the present work it has been found possible to extract the volatile fatty acids from plasma, as their sodium salts, with isopropyl alcohol, thereby eliminating the need for steam distillation.

Previous techniques for the determination of the acids after extraction have used either paper or gas - liquid chromatography. The paper-chromatographic methods have depended on the production of a coloured derivative of the acids, which can be determined by densitometry. Tranger[3] prepared the hydroxamates from volatile fatty acid methyl esters and determined them by measuring the intensity of the colour formed with iron(III) chloride. Considerable losses were reported when using this method. The gas - liquid chromatographic analyses of volatile fatty acids have generally been carried out in dilute solutions of mineral acid, especially metaphosphoric acid, and it is very difficult to obtain good results with such methods.[11] Ghosting[12] and anomalous peak broadening[13] have been observed, and poor peak shapes are frequently obtained.[14]

In order to overcome these difficulties, volatile fatty acid derivatives have been produced and determined by gas - liquid chromatographic methods.[15,16] Preliminary efforts, made in this laboratory, to apply these techniques to the volatile fatty acids in blood have been unsuccessful, probably because the minute amounts of acids present make it difficult to convert all of the acid into the derivative. A simple way of overcoming the problem of the gas - liquid chromatographic analysis of solutions of volatile fatty acids is to inject them on to the column in an organic acid. Zerilli, Brambilla and Rimorini[9] have suggested 9 + 1 acetone - formic acid as a suitable solvent system. In the present work 9 + 1 diethyl ether - formic acid has been found to give sharper peaks and less solvent tailing.

EXPERIMENTAL

REAGENTS—

Formic acid (*Analar*)—This acid was fractionally distilled from a 500-ml flask by using a 0·5 m × 20 mm lagged glass column, packed with Fenske helices and fitted with a partial take-off head. The first 40 ml of distillate collected were redistilled in semi-micro apparatus and the first 10 ml of distillate collected were again fractionally distilled in the same apparatus. The first 2 ml of this distillate were found to be pure on gas - liquid chromatographic analysis and contained no traces of acetic, propionic and butyric acid, which had been present in the original volume of formic acid.

Isopropyl alcohol—Used after fractional distillation with a 1-m Vigreux column.

Diethyl ether (*May and Baker Ltd., Pronalys*)—Used without further purification.

Samples of acetic, propionic, isobutyric, butyric, isovaleric (3-methylbutanoic) and valeric acids were supplied by BDH Chemicals Ltd. and were of the highest purity available.

n-Hexanoic acid (*Sigma Chemical Co. Ltd.*)—This reagent was better than 99 per cent. pure, and gas - liquid chromatographic analysis indicated that it contained none of the C_2 to C_5 volatile fatty acids. As preliminary investigation had indicated that n-hexanoic acid was

not present in appreciable amounts in sheep or bovine plasma it was chosen as an internal standard for the method. Aqueous solutions of the acid were made up to contain 10^{-2} mg ml^{-1} and were used as described in the following section.

METHOD

SAMPLE EXTRACTION AND PREPARATION FOR GAS - LIQUID CHROMATOGRAPHY—

Take a 10-ml sample of blood and centrifuge it at 3500 r.p.m. for 15 minutes. Transfer, by pipette, 6·0 ml of the supernatant plasma into another centrifuge tube and add 1·0 ml of the n-hexanoic acid standard solution. The volumes of sample and internal standard must be accurately measured. Adjust the mixture to pH 9 to 10 by adding 0·05 M sodium hydroxide solution dropwise, then add 40 ml of redistilled isopropyl alcohol and remove the protein precipitate by centrifugation at 3500 r.p.m. for 30 minutes. Decant the supernatant liquid and evaporate it to dryness on a rotary film evaporator, the final 1 ml of solution being taken to dryness in a small (10 ml) B14 test-tube. Seal the top of this tube with a layer of Parafilm (Gallenkamp Co. Ltd.) and inject 200 μl of freshly mixed 9 + 1 ether - formic acid (triple distilled) through the film with a syringe. Seal the resultant hole immediately with another layer of Parafilm. Agitate the contents of the tube vigorously on a Whirlimixer and allow them to stand for 15 minutes. Finally, withdraw 10 μl of the solution from the tube and inject it on to the gas - liquid chromatographic column.

GAS CHROMATOGRAPHY—

A Pye Unicam, Model 104, dual-column gas chromatograph, fitted with flame-ionisation detectors, was used, and separations were carried out by using glass columns that were 214 cm long by 6 mm o.d., packed with 17 per cent. neopentyl glycol adipate (Phase Separations Ltd.) in 3 per cent. orthophosphoric acid supported on Embacel (May and Baker Ltd.) of 60 to 100 mesh (R. S. Reid, personal communication). Condition the packed column at 150 °C overnight. For volatile fatty acid analysis use the following conditions: column temperature, 105 °C; detector temperature, 150 °C; and nitrogen flow-rate, 60 ml min^{-1}. (Optimise the hydrogen and air flow-rates for maximum response.) Inject the mixture of acids directly on to the column packing. Under these conditions typical retention times in minutes of the acids are (retention times relative to n-hexanoic acid in parentheses): acetic, 3·13 (0·095); propionic, 5·5 (0·167); isobutyric, 7·0 (0·212); butyric, 9·38 (0·284); isovaleric, 12·5 (0·379); valeric, 17·5 (0·53); and n-hexanoic, 33·0 (1·0).

PREPARATION OF CALIBRATION GRAPH—

Aqueous solutions of each of the C_2 to C_5 volatile fatty acids were prepared in a range of concentrations in the expected physiological ranges (*i.e.*, acetic acid from 1 to 4 mg per 100 ml and the other acids from 0·01 to 0·04 mg per 100 ml). These solutions were made up in calibrated glassware and standard mixtures of the acids were prepared from them in order to calibrate the method. Known amounts of each mixture were then taken through the analytical procedure exactly as a plasma sample would have been. The peak areas given by the individual acids were measured relative to that of the internal standard. The use of peak height × width at half-height as an estimate of peak area was found to give the closest results to cutting and weighing the chart paper and the former method was used.

RESULTS AND DISCUSSION—

Fig. 1 shows the calibration graph obtained for acetic acid and Fig. 2 those for propionic, isobutyric, butyric, isovaleric and valeric acids. Each graph approximates to a straight line and it was possible to determine a calibration factor for each acid that was the slope of the line in the calibration graph. It was then possible to calculate the original concentration of each acid in every blood sample, knowing the area of each peak and the amount of internal standard that had been added, by applying the following equation:

$$\text{Mass of acid} = \frac{\text{Peak area of acid} \times \text{mass of n-hexanoic acid}}{\text{Peak area of n-hexanoic acid} \times \text{calibration factor}} \quad \ldots \ldots \quad (1)$$

A typical chromatogram of a sample of sheep plasma is shown in Fig. 3. Four experiments were carried out in order to test the completeness of recovery of known amounts of

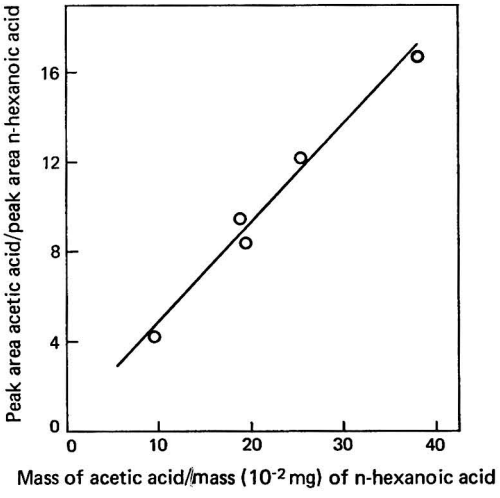

Fig. 1. Calibration graph for determination of acetic acid. Slope = 0·44

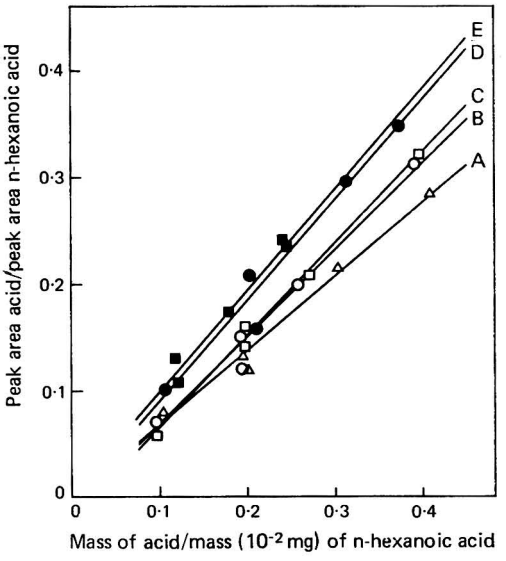

Fig. 2. Calibration graph for determination of propionic (A), isobutyric (B), butyric (C), isovaleric (D) and valeric (E) acids. Slopes are: A, 0·70; B, 0·83; C, 0·86; D, 0·95; and E, 0·95

added volatile fatty acids from plasma. Four separate plasma samples were each divided into two equal portions and one portion of each was used to determine the amounts of the individual volatile fatty acids present initially. A measured amount of a standard aqueous mixture of the acids was added to the remaining portion of each plasma sample and the resulting solutions were analysed. It was then possible to calculate the recovery of each acid, knowing the initial and final amounts. Results are shown in Table I as the individual acids present before and after addition of the standard mixtures.

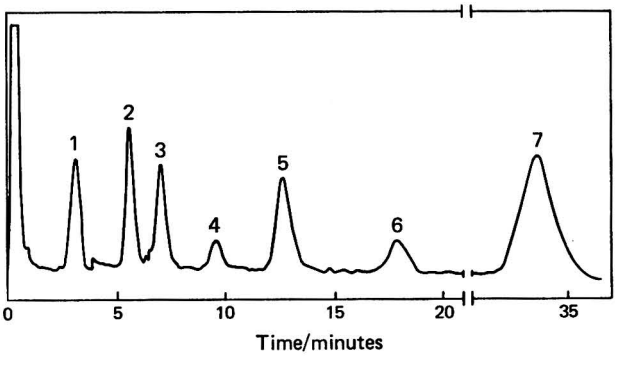

Fig. 3. A typical chromatogram of a sample of sheep hepatic portal vein plasma showing: 1, acetic; 2, propionic; 3, isobutyric; 4, butyric; 5, isovaleric; 6, valeric; and 7, n-hexanoic acid peaks. Attenuation factors: 1, ×2000; 2, ×500; 3, ×100; 4, ×200; 5, ×100; 6, ×50; and 7, ×200

A blank performed on 10 ml of distilled water contained no detectable amounts of any of the C_2 to C_5 acids and the results can therefore be taken as a measure of the accuracy of the method. The mean recoveries all lie in the range 92 to 106 per cent. There are evidently no substantial losses, except of propionic acid, but in this instance the mean is probably

TABLE I

RECOVERY OF KNOWN AMOUNTS OF VOLATILE FATTY ACIDS ADDED TO PLASMA

	Acetic acid/mg	Propionic acid/mg	Isobutyric acid/mg	Butyric acid/mg	Isovaleric acid/mg	Valeric acid/mg
Original amount ..	0·182	0·0037	0·0003	0·0027	0·0006	0·0008
Amount added ..	0·210	0·0020	0·0020	0·0019	0·0019	0·0013
Total amount recovered	0·380	0·0058	0·0025	0·0051	0·0027	0·0018
Recovery, per cent. ..	96·9	101·7	108·7	110·8	108·0	85·7
Original amount ..	0·146	0·0017	0·0002	0·0012	0·0003	0·0003
Amount added ..	0·210	0·0020	0·0020	0·0019	0·0019	0·0013
Total amount recovered	0·362	0·0035	0·0024	0·0029	0·0024	0·0015
Recovery, per cent. ..	101·7	94·6	109·0	93·5	109·0	93·8
Original amount ..	0·168	0·0045	0·0006	0·0025	0·0008	0·0006
Amount added ..	0·420	0·0041	0·0039	0·0039	0·0037	0·0026
Total amount recovered	0·575	0·0082	0·0041	0·0065	0·0049	0·0035
Recovery, per cent. ..	97·8	95·3	91·1	103·1	108·9	109·4
Original amount ..	0·185	0·0049	0·0015	0·0017	0·0008	0·0004
Amount added ..	0·420	0·0041	0·0039	0·0039	0·0037	0·0026
Total amount recovered	0·580	0·0070	0·0057	0·0060	0·0044	0·0034
Recovery, per cent. ..	95·9	77·8	105·5	107·2	97·8	112·3
Mean recovery, per cent.	98·1	92·3	103·6	103·7	105·9	100·6
Standard deviation ..	2·5	10·3	8·5	7·5	5·4	13·0

artificially low, owing to the final recovery figure of 77·8 per cent. The over-all accuracy of the method could possibly be increased by the use of an integrator for the measurement of peak area.

CONCLUSION

The method described in this paper for the determination of volatile fatty acids in plasma has been shown to be quantitative and reproducible for the peripheral blood of ruminants. It has the distinct advantage, over published methods involving steam distillation, of relative speed and good recoveries. The gas-chromatographic technique is accurate and reliable and, after several months of continuous use, there is no evidence of column deterioration or the build-up of unstable products.

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Received *October 8th*, 1973
Accepted *December 31st*, 1973

Quantitative Determination of the Enantiomeric Purity of Synthetic Pyrethroids

Part II.* *S*-Bioallethrin

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S-Bioallethrin consists primarily of (+)-allethronyl-(+)-*trans*-chrysanthemate, but technical samples contain small amounts of other allethrin isomers. The ratio of diastereoisomers can be measured directly from the nuclear magnetic resonance spectrum after using a europium shift reagent, when many of the resonances split into two distinct diastereoisomer signals. If the enantiomeric purity of the chrysanthemate moiety is determined independently, then the absolute enantiomeric purity of the allethrin sample can be calculated. Standard deviations for the measurement of laboratory or technical samples were between 0·3 and 0·7 per cent.

The accuracy of the method was verified by independently determining the enantiomer ratios of various allethrolone samples by gas chromatography of their diastereoisomeric (−)-α-methoxy-α-trifluoromethylphenylacetic esters, esterifying with natural (+)-*trans*-chrysanthemic acid and redetermining the enantiomeric purity by nuclear magnetic resonance. Good agreement was achieved between the two determinations.

The nuclear magnetic resonance method should also be suitable for measuring the diastereoisomer ratio of *cis*-allethrins.

The chrysanthemate allethrin, which is an insecticide, can occur in eight isomeric forms; the chrysanthemic acid moiety exhibits both optical and geometrical isomerism and the allethrolone moiety is optically active.

This insecticide was introduced in 1949[1] as the racemic mixture of *cis*- and *trans*-chrysanthemates, in the approximate ratio 25:75. Greater insecticidal activity has since been obtained by resolving the acid into the (+)-*trans* form (bioallethrin†)[2–4] and the compound is now becoming commercially available with the allethrolone also resolved into the (+)- or *S*-enantiomer, under the name *S*-bioallethrin or Esbiol‡ [(+)-allethronyl-(+)-*trans*-chrysanthemate].

Polarimetry is a useful method for checking the composition of commercial *S*-bioallethrin samples as the isomer with the highest insecticidal activity also exhibits the highest negative rotation[5] (the values originally published by LaForge, Green and Schechter[6,7] have proved to be inaccurate). However, the method can give only an indication of the minimum amount of (+)-allethronyl-(+)-*trans*-chrysanthemate present in the sample without defining the other constituents. A more specific method of determining the enantiomeric purity is therefore desirable.

An earlier paper[8] described a method for determining the enantiomeric purity of *cis* or *trans* forms of the chrysanthemic acid moiety by hydrolysis of the allethrin and analysis by gas chromatography of the diastereoisomers formed by reaction of the (+)- and (−)-acids with (+)-α-methylbenzylamine. Analogous methods cannot be used for the allethrolone moiety because it is not possible to recover the free alcohol by hydrolysis of allethrin unless the semicarbazone is first prepared.[9] The method described here provides a means of directly measuring the ratio of diastereoisomers in laboratory and technical *S*-bioallethrin samples, by use of nuclear magnetic resonance spectroscopy, from which the enantiomeric purity of the allethrolone can readily be calculated.

The nuclear magnetic resonance spectra of some natural cyclopropanes with lanthanide shift reagents has been described by Crombie, Findley and Whiting.[10]

* For details of Part I of this series, see reference list, p. 337.

† Proposed B.S.I. approved name.

‡ Registered trade name, Roussel Uclaf S.A., Romainville, France.

Experimental

Reagents—

Bioallethrin—(±)-Allethronyl-(+)-*trans*-chrysanthemate, technical grade.

S-*Bioallethrin*—(+)-Allethronyl-(+)-*trans*-chrysanthemate. Both laboratory prepared and technical samples were used, the latter supplied by Roussel Uclaf S.A. under the trade name Esbiol.

α-(±)-trans-*Allethrin*—A crystalline racemate of (−)-allethronyl-(+)-*trans*-chrysanthemate and (+)-allethronyl-(−)-*trans*-chrysanthemate, melting-point 50 to 51 °C, prepared by the method of Schechter, LaForge, Zimmerli and Thomas.[11]

S-*Allethrolone*—One sample was kindly supplied by Dr. M. Elliott of the Rothamsted Experimental Station, Harpenden, Herts. Other samples were prepared by hydrolysis of *S*-bioallethrin semicarbazones by using established procedures[12,13] and were blended with (±)-allethrolone (Benzol Products Inc., Edison, New Jersey).

(+)-*Pyrethrolone*—This was supplied by Dr. M. Elliott of Rothamsted Experimental Station.

(−)-trans-*Chrysanthemoyl chloride*—Boiling-point 82 °C (0·5 mm Hg); $[\alpha]_D^{22}$ − 24·6° (14·5 per cent. in 2,2,4-trimethylpentane). The chloride was prepared from (+)-*trans*-chrysanthemic acid that had been obtained by hydrolysis of pyrethrum extract.[14]

Shift reagent, *Eu(fod-*d_9*)*$_3$—Tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-d_6-octane-4,6-dione-d_3) europium(III) (from Nuclear Magnetic Resonance Ltd., High Wycombe, Bucks).

Carbon tetrachloride (*nuclear magnetic resonance grade*)—Uvasol (E. Merck), dried over type 3A molecular sieve.

(−)-α-*Methoxy-α-trifluoromethylphenylacetyl chloride* [(−)-*MTPA chloride*]—$[\alpha]_D^{24}$ − 129° (5·0 per cent. in carbon tetrachloride); boiling-point 99 °C (12 mm Hg); prepared from (−)-MTPA, $[\alpha]_D^{20}$ − 72° (neat) (Ralph N. Emanuel Ltd., Wembley, Middlesex) using published methods.[15]

(−)-*Menthol*.

Nuclear magnetic resonance spectra were recorded on a Varian T-60 spectrometer by use of high-resolution tubes with tetramethylsilane as internal standard.

Nuclear magnetic resonance measurement of the diastereoisomer ratio of *S*-bioallethrin—

The sample (50 mg, 96 to 100 per cent. in total esters) and Eu(fod-d_9)$_3$ (200 mg) were dissolved in the carbon tetrachloride (0·4 ml) and filtered into a nuclear magnetic resonance spectrometer tube. Signals for the α- and β-diastereoisomers of the cyclopropane $^{\bullet}CH_3$ (see below) were found at 3·57 and 3·10 p.p.m. downfield of tetramethylsilane, respectively. Peak areas were recorded on a sweep width of 250 Hz and the average of five integrams (each of 50 s sweep time) was taken.

Gas - liquid chromatographic determination of allethrolone enantiomers—

(−)-MTPA chloride (22 mg) in 2 ml of dry benzene was added slowly (over 5 minutes) at room temperature to a stirred solution of allethrolone (10 mg) and dry pyridine (5 μl), also in 2 ml of dry benzene. The mixture was stirred at room temperature for 1 hour, then refluxed for 2 hours and evaporated *in vacuo* at 30 °C to a volume of approximately 1 ml. This product was purified by chromatography on 1 g of Woelm neutral aluminium oxide (Brockmann activity III) contained in a Pasteur pipette and eluted with 15 ml of benzene. The solvent was evaporated *in vacuo* and the residue made up to 10 ml with ethyl acetate in preparation for gas - liquid chromatographic analysis, which was performed on a Pye, Series 104, gas chromatograph fitted with a flame-ionisation detector and using a 20 foot × $\frac{1}{8}$ inch glass column packed with 6 per cent. LSX-3 on 100 to 120 mesh Gas-Chrom Q. The column oven temperature was 210 °C and the carrier gas (nitrogen) flow-rate 8·5 ml min^{-1}.

Retention times were: for (−)-allethronyl-(−)-MTPA, 70·5 minutes; and for (+)-allethronyl-(−)-MTPA, 74·1 minutes.

Enantiomeric purity of (−)-MTPA—

A solution of 8 mg of (−)-menthol, 10 mg of (−)-MTPA chloride and 50 μl of dry pyridine in 3 ml of dry benzene was refluxed for 2 hours, evaporated *in vacuo* and made up

to 5 ml with ethyl acetate. The menthyl esters were analysed on a 50 m × 0·2 mm glass capillary column coated with free fatty acid phase (FFAP), operated under the following conditions: column temperature, 163 °C; injector temperature, 209 °C; carrier gas, nitrogen at 14 p.s.i. inlet pressure.

The solution (1 μl) was injected, using an inlet split ratio of approximately 100:1. The preparation of the column and the equipment used have been described previously.[8] Retention times were: (−)-menthyl-(−)-MTPA, 36·4 minutes; and for (−)-menthyl-(+)-MTPA, 37·1 minutes.

Reconstitution of *S*-bioallethrins—

(−)-*trans*-Chrysanthemoyl chloride (230 mg), dissolved in 5 ml of dry benzene, was added dropwise over 15 minutes to the *S*-allethrolone (150 mg) and 0·1 ml of dry pyridine in 5 ml of dry benzene, stirred at 0 °C. The mixture was stirred at room temperature overnight, diluted with 20 ml of diethyl ether, washed with 10-ml portions of 2 M hydrochloric acid, water, saturated sodium hydrogen carbonate solution, then water again and dried over anhydrous calcium sulphate. The solvent was evaporated *in vacuo* to approximately 1 ml and the product purified by chromatography on Woelm neutral aluminium oxide (Brockmann activity III), being eluted with 25 per cent. ether in n-hexane.

Results and discussion

Diastereoisomers often exhibit chemical shift differences in their nuclear magnetic resonance spectra, particularly where the protons are close to the asymmetric centres.[16,17] It has previously been observed[18] that synthetic pyrethroids prepared from racemic alcohols show diastereoisomeric non-equivalence for one of the cyclopropane methyl groups. The spectrum of bioallethrin recorded at 60 MHz in solution in carbon tetrachloride shows evidence of diastereoisomeric non-equivalence for three signals [Fig. 1 (*a*) and 1 (*b*)], at 1·25 p.p.m. (cyclopropane $^{a}CH_3$), 1·98 p.p.m. (cyclopentenolone $^{1}CH_3$) and 2·25 p.p.m. (cyclopentenolone ^{1}H) but the separations are not sufficient to allow quantitative determination of the diastereoisomer ratio. With the addition of a europium shift reagent the diastereoisomers are differentially shifted to lower field, causing many of the resonances to split into two separate diastereoisomer signals [Fig. 2 (*a*)]. The nuclear magnetic resonance spectrum of allethrin, obtained by using a shift reagent, has recently been published by Sugiyama *et al.*[19]

The optimum conditions for the quantitative measurement of the diastereoisomer ratio are obtained by using 50 mg of allethrin sample and 200 mg of Eu(fod-d_9)$_3$ dissolved in 0·4 ml of carbon tetrachloride, thus giving a mole ratio of pyrethroid to shift reagent of 0·88:1. With these concentrations the cyclopropane $^{a}CH_3$ gives two signals, at 3·57 p.p.m. and 3·10 p.p.m. for the α- and β-diastereoisomers, respectively. The β-diastereoisomer peak consists of the two enantiomers, (+)-allethronyl-(+)-*trans*-chrysanthemate and (−)-allethronyl-(−)-*trans*-chrysanthemate, while the α-diastereoisomer comprises the (+) (−) and (−) (+) forms [*cf.*, α-($\pm$)-*trans*-allethrin]. The $^{a}CH_3$ peaks are ideal for quantitative measurement, being intense singlets well separated from interfering resonances. The assignments were confirmed by comparing the spectra of bioallethrin [Fig. 2 (*a*)], *S*-bioallethrin [Fig. 2 (*b*)] and α-($\pm$)-*trans*-allethrin [Fig. 2 (*c*)].

Measurement of the diastereoisomer ratio does not, of course, give a direct result for the enantiomeric purity of the sample. The (+) to (−) ratio of the chrysanthemic acid moiety can be determined independently[8] and, with the nuclear magnetic resonance measurements described above, the enantiomeric purity of the allethrolone can be calculated by applying the following equation:

$$S\text{-allethrolone, per cent.} = \frac{100\ (P + x - 100)}{2x - 100} \quad .. \quad .. \quad .. \quad (1)$$

and hence

$$S\text{-bioallethrin, per cent.} = \frac{x\ (P + x - 100)}{2x - 100} \quad .. \quad .. \quad .. \quad (2)$$

where P is the observed percentage of the β-diastereoisomer peak and x is the percentage of (+)-enantiomer in the *trans*-chrysanthemic acid.*

In practice this calculation may not be necessary, for if the chrysanthemic acid is of high enantiomeric purity and the allethrolone is predominantly in the (+) form, then the amount of (−)-allethronyl-(−)-*trans*-chrysanthemate present must be low and the β-diastereoisomer peak will give an approximate measure of the *S*-bioallethrin content.

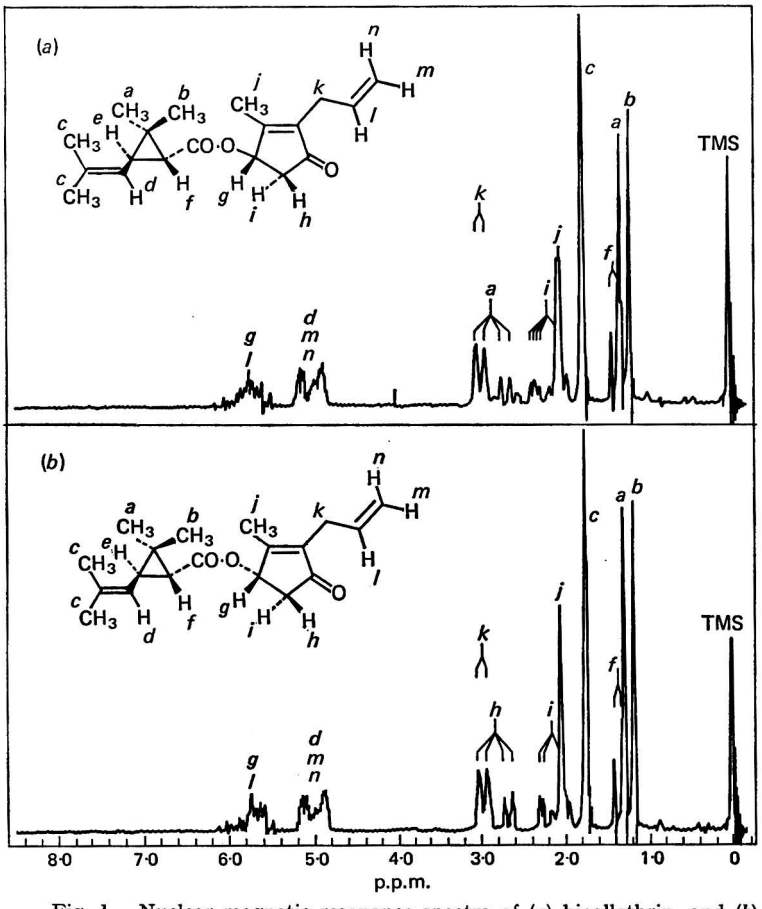

Fig. 1. Nuclear magnetic resonance spectra of (*a*) bioallethrin, and (*b*) *S*-bioallethrin recorded in solution in carbon tetrachloride without shift reagent. Assignments according to Bramwell *et al.*[18]

An optically pure sample of *S*-bioallethrin was not available for use as a standard to check the accuracy of the nuclear magnetic resonance method. Instead, samples of allethrolone were independently analysed for (+) to (−) ratio, esterified with natural (+)-*trans*-chrysanthemic acid and used as secondary nuclear magnetic resonance standards.

(−)-α-Methoxy-α-trifluoromethylphenylacetic acid [(−)-MTPA] is an established reagent for determining the enantiomeric purity of alcohols by nuclear magnetic resonance spectroscopy.[15,20] The (−)-MTPA esters of (±)-allethrolone give two signals, separated by 8·5 Hz, for the diastereoisomeric cyclopentenolone $^1CH_3$ [Fig. 3 (*a*)]; no shift reagents were required in this instance. The lower field resonance at 2·03 p.p.m. was assigned to the (+)-allethronyl ester by comparison with the (−)-MTPA ester of natural (+)-pyrethrolone [Fig. 3 (*b*)]; the configurations of the groups around the cyclopentenolone ring are identical in the two compounds,[21] the only difference being in the lengths of the side chains.

* In the derivation of this equation it is assumed that the enantiomers of acid and alcohol are randomly distributed, *i.e.*, that no kinetic resolution has taken place during the esterification stage.

Although the nuclear magnetic resonance signals were sufficiently well separated for quantitative measurement, an alternative and preferred method for measuring the diastereoisomer ratio was by gas chromatography of the (—)-MTPA esters on a 20-foot glass column packed with 6 per cent. LSX-3 on Gas-Chrom Q support. The resolution obtained

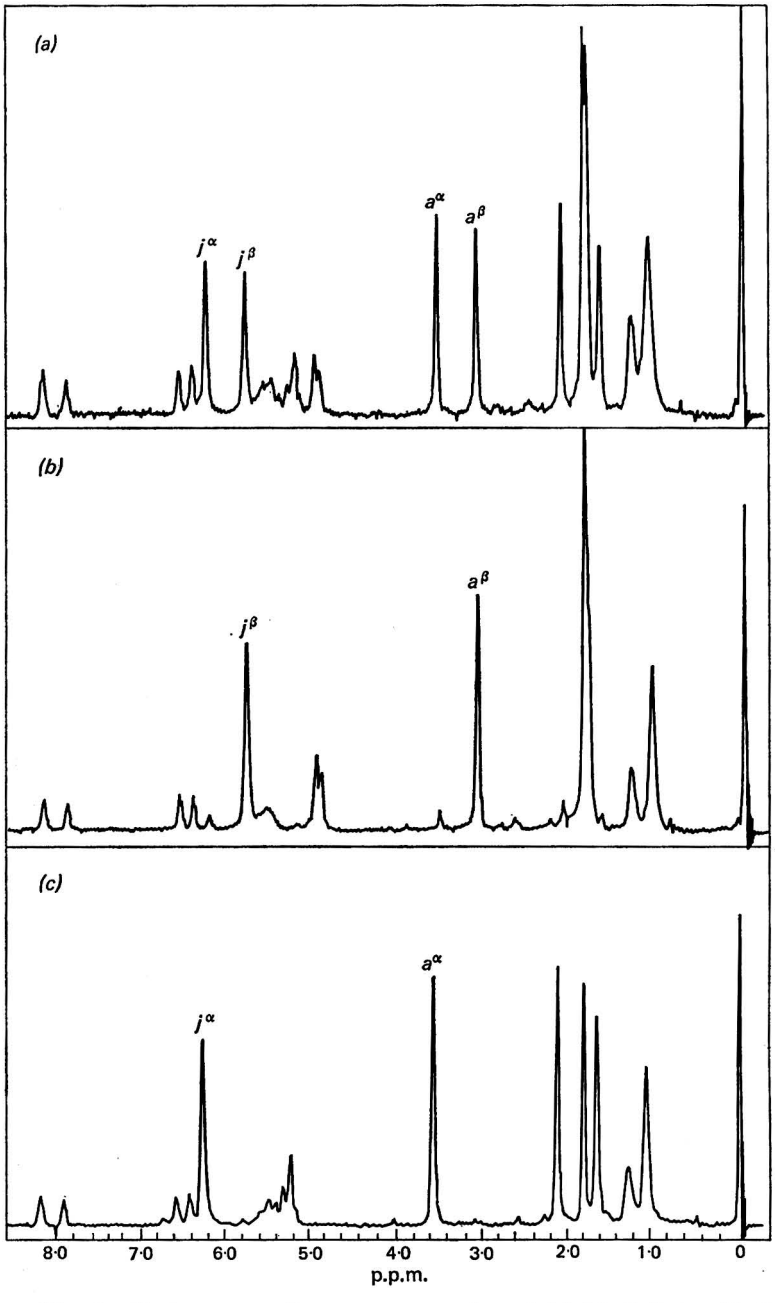

Fig. 2. Nuclear magnetic resonance spectra of (*a*) bioallethrin, (*b*) *S*-bioallethrin, and (*c*) α-(±)-*trans*-allethrin with shift reagent

between the diastereoisomers was 1·45, only marginally above the base-line. Duplicate samples of allethrolone containing 50 to 91 per cent. of (+)-enantiomer were esterified with (−)-MTPA chloride and each was analysed twice by this procedure. Peak areas were measured by use of a disc integrator and the standard deviations calculated from the four analyses of each sample were 0·3 per cent.

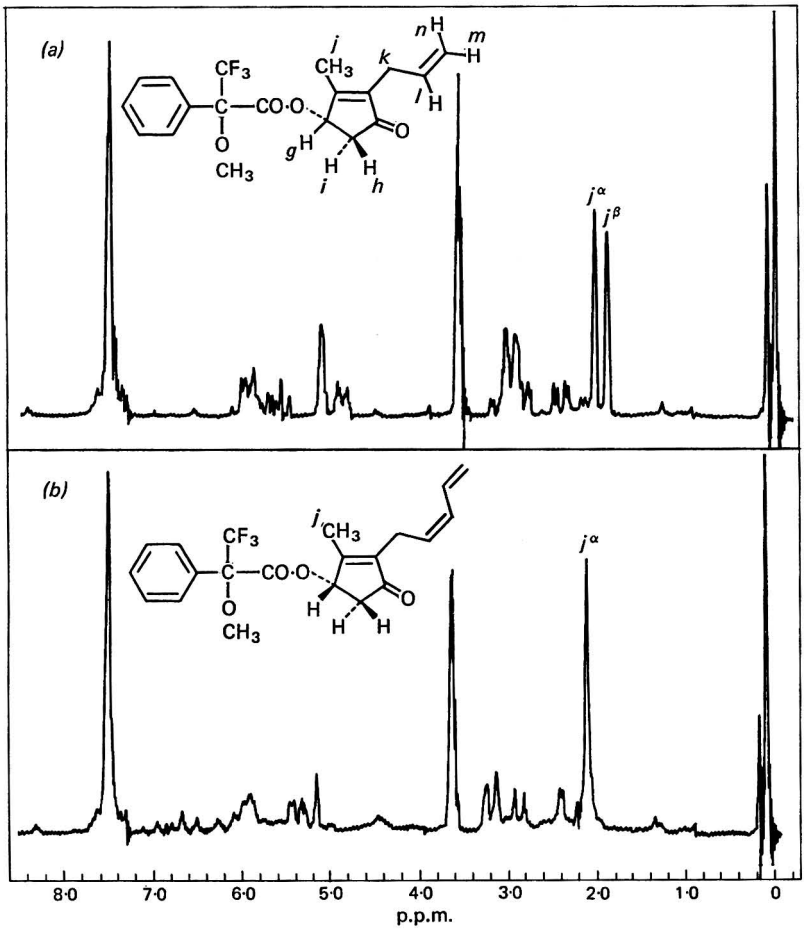

Fig. 3. Nuclear magnetic resonance spectra of the (−)-α-methoxy-α-trifluoromethylphenylacetic esters of (*a*) (±)-allethrolone and (*b*) (+)-pyrethrolone

As the (−)-MTPA is a synthetic rather than naturally derived chemical it was considered pertinent to check the enantiomeric purity of the batch used for these determinations. This was accomplished by analysing the diastereoisomeric (−)-menthyl esters on a 50 m × 0·25 mm glass capillary column coated with FFAP. A base-line separation between the diastereoisomers was obtained and from four determinations the ratio of (−)-MTPA to (+)-MTPA was found to be 99·3:0·7 with a standard deviation of 0·2.

The enantiomer ratios of the allethrolone samples were corrected for the small amount of (+)-MTPA present in the acid forming the derivatives by using equation (1) where, in this instance, P was the observed percentage of the (+)-allethronyl-(−)-MTPA gas - liquid chromatographic peak and x the percentage of (−)-MTPA in the acid. The corrected values are given in Table I.

S-Bioallethrin samples were reconstituted from the allethrolones by use of natural (+)-*trans*-chrysanthemic acid and the (+) to (−) ratios determined by the nuclear magnetic

TABLE I

PERCENTAGE OF (+)-ENANTIOMER IN ALLETHROLONE SAMPLES DETERMINED BY GAS - LIQUID CHROMATOGRAPHY AND NUCLEAR MAGNETIC RESONANCE METHODS

(+)-Allethrolone by g.l.c. of (−)-MTPA esters, per cent.	(+)-Allethrolone by n.m.r. of (+)-*trans*-chrysanthemates, per cent.
50·1	50·0
76·5	77·0
87·7	87·5
91·5	91·4
91·9	92·3

resonance method. (As, in this instance, the acid is enantiomerically pure, the diastereoisomer ratio is equal to the enantiomer ratio of the alcohol.) The results given in Table I are the average of five integrations; standard deviations were between 0·3 and 0·7 per cent. The enantiomer ratios of the allethrolone samples determined by the nuclear magnetic resonance method thus compared well with the gas - liquid chromatographic values, all of the differences being less than the combined standard deviations.

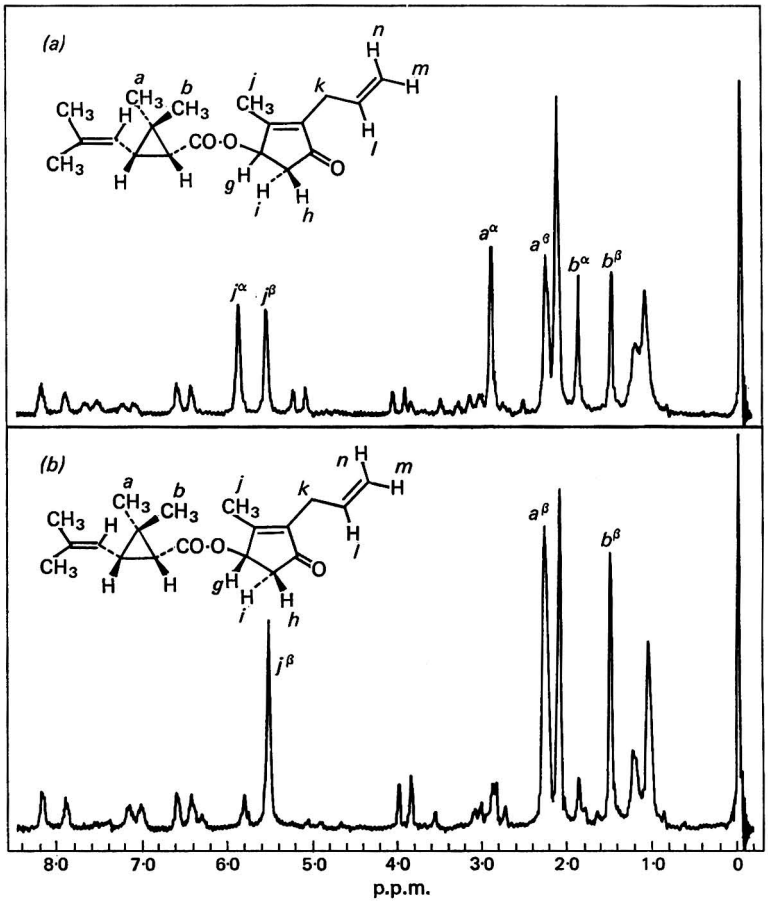

Fig. 4. Nuclear magnetic resonance spectra of *cis*-allethrins with shift reagent. (*a*), (±)-Allethronyl-(+)-*cis*-chrysanthemate and (*b*), (+)-allethronyl-(+)-*cis*-chrysanthemate

Other constituents of technical *S*-bioallethrin are esters of *cis*-chrysanthemic acid and free chrysanthemic acid (each comprising generally less than 2 per cent. of the total). When using equivalent amounts of shift reagent, neither compound gave peaks lying within the area from 3·0 to 3·6 p.p.m. that was used for these measurements. No increases in the standard deviations were observed for measurements of technical *S*-bioallethrin samples; multiple scan accumulation did not significantly improve precision.

The method would also appear to be suitable for determining the diastereoisomer ratio of *cis*-allethrins. Fig. 4 (*a*) and (*b*) shows the nuclear magnetic resonance spectra, after treatment with the shift reagent, of (±)-allethronyl-(+)-*cis*-chrysanthemate and (+)-allethronyl-(+)-*cis*-chrysanthemate, respectively (the latter being contaminated with approximately 13 per cent. of the (−)-allethronyl isomer). The cyclopropane $^{a}CH_3$ peaks at 2·88 p.p.m. and 2·27 p.p.m. cannot be used in this instance because the latter overlaps with the methyl peaks of the isobutenyl groups. However, signals from both $^{1}CH_3$ and $^{b}CH_3$ are fully resolved and either should be suitable for quantitative measurement.

The authors are grateful to Dr. M. Elliott of Rothamsted Experimental Station for the gift of valuable materials.

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NOTE—Reference 8 is to Part I of this series.

Received *October 24th*, 1973
Accepted *November 28th*, 1973

Analyst, June, 1974, Vol. 99, *pp.* 338–354

The Detection and Determination of Polynuclear Aromatic Hydrocarbons by Luminescence Spectrometry Utilising the Shpol'skii Effect at 77 K

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The luminescence emission spectra of twenty-three polynuclear aromatic hydrocarbons (PAH) have been examined in n-alkane solvents at 77 K. The Shpol'skii effect, in which narrow-band (quasi-linear) emission spectra are obtained under these conditions when a monochromator of adequate resolving power is used, is shown to be readily observed for twelve of the compounds examined in these solvents. Quasi-linear emission spectra have also been obtained in tetrahydrofuran for some of the PAH compounds examined. These emission spectra provide for unambiguous qualitative identification of PAH compounds at trace concentrations in solution; this effect is demonstrated by identification of the compounds present in an eight-component mixture of PAH compounds.

Measurement of the low-temperature quasi-linear luminescence intensity can be applied quantitatively to the determination of these compounds provided that a standard additions procedure is employed in conjunction with the use of an internal standard to ensure sufficient accuracy and precision.

THE detection and determination of trace concentrations of polynuclear aromatic hydrocarbons (PAH) is of extreme importance as most of these compounds are toxic and many are carcinogenic.[1] The development of methods for their unambiguous identification and accurate determination in samples of air, water, foods and petroleum products and effluents is therefore necessary. Methods based on solution spectrofluorimetry and spectrophosphorimetry have been widely employed for these purposes.[2–6] One of the difficulties with these techniques arises from the relatively broad-band excitation and emission spectra observed for PAH compounds in many solvents at room temperature and even at low temperature in those solvents which form optically transparent glasses that are suitable for luminescence spectrometry with right-angle illumination.

As a consequence, methods for the determination of particular PAH compounds by luminescence spectrometry under these conditions may suffer interference caused by the overlapping excitation or emission spectra of other similar compounds present in the sample. It is usually necessary, therefore, to resort to preliminary separation of the compound by chromatography or extraction before determining it by fluorimetry or phosphorimetry. In complex PAH mixtures, such separations are frequently necessary before even qualitative identification of individual compounds present can be made by these techniques.

A further problem arises from the fact that most commercially available fluorescence spectrometers utilise relatively low-resolution, high-aperture monochromators to permit detection of the weak luminescence emission obtained with trace concentrations of the species to be determined. Thus, even if structured luminescence emission is present, it may be difficult to observe with the spectral resolution attainable with this type of monochromator.

In 1952, Shpol'skii, Il'ina and Klimova[7] reported that some aromatic compounds, when included in the crystalline matrix formed at 77 K or below by use of selected n-alkane solvents, exhibited extremely well resolved fine structure in their luminescence emission spectra. This phenomenon was confirmed by Bowen and Brocklehurst.[8] The observation under these conditions of line-like structure, in which individual lines may be less than 0·1 nm in half-width, can be explained by the postulate that the solute analyte molecules become embedded in the crystalline solvent lattice formed on cooling. The solute molecules are thus held in strictly oriented positions and at low concentrations are separated by large distances so that

they do not interact. In contrast to the case that applies in solvents which form transparent glasses at low temperature, where the glass does not show a short-range order and the electronic transitions are very sensitive to variation in the molecular field, in the crystalline solid solutions produced for PAH compounds in n-alkane solvents the solute molecules experience a well defined molecular field that gives rise to sharp-line (quasi-linear) electronic spectra.[9] The spectra exhibit the vibrational frequencies of the centres in the ground state; several workers have shown that good correlation can be achieved with results obtained independently from infrared or Raman spectra.[10–12] The quasi-linear spectra of more than 100 organic compounds have been recorded and the application of the technique has been reviewed.[13–17]

It has been observed that the molecular dimensions of the n-alkane solvent used must be matched to those of the solute molecule in order to obtain well defined quasi-linear spectra.[16] The early studies of the Shpol'skii effect indicated that measurement of the quasi-linear spectra obtained at low temperature for PAH and other compounds should provide a powerful tool for fundamental investigation of molecular structure and for the sensitive, and extremely selective, detection and determination of these compounds. Despite the predictions of advantages to be gained by the application of the Shpol'skii effect, which were recorded in the early literature, Winefordner and Lucasiewicz[18] have commented adversely on its potential and outlined possible difficulties associated with its application to quantitative analysis. These workers have also stated that few papers have presented a detailed description of the use of the technique in a specific analytical method and which reported the usual data pertaining to reproducibility, accuracy and precision, and that the availability of suitable commercial luminescence instrumentation severely limits its applications.

While those papers which have been concerned with the Shpol'skii effect may in many instances lack adequate reproducibility, accuracy and precision data, a considerable number of publications have described the application of the quasi-linear luminescence measurements to both qualitative and quantitative examination of real samples. Thus Il'ina and Personov[19] have applied the technique to the detection of 1,12-benzoperylene in Jurassic and Cretaceous sedimentary rocks and perylene in tertiary sediments.

Dikun[20] identified pyrene, 1,2-benzopyrene, 3,4-benzopyrene, perylene, 1,12-benzoperylene and 1,2,7,8-dibenzanthracene in smoked fish and polluted air by using their quasi-linear spectra in n-hexane solvent at 77 K; with the same technique, he also detected *o*-phenylenepyrene, 1,2,4,5-dibenzopyrene and 3,4,9,10-dibenzopyrene in polluted air. Gurov and Novikov[21] identified anthracene, pyrene, 3,4-benzopyrene, 1,12-benzoperylene, perylene and coronene in soil and snow samples using the quasi-linear luminescence spectra obtained at 77 K in n-hexane solvent. Parker and Hatchard[22] have applied the technique using n-octane - cyclohexane solvent to an investigation of an unusual photo-reaction of 3,4-benzopyrene in solutions containing polymer.

Eichhoff and Köhler[23] determined 3,4-benzopyrene in the atmosphere by a method based on direct measurement of the absolute intensity at 403·0 nm of its quasi-linear emission in n-heptane at 79 K. Personov[24] determined 3,4-benzopyrene by using the quasi-linear emission of coronene for internal standardisation in order to avoid random errors caused by variations in experimental conditions, and Dikun[25] devised a similar method for determining 3,4-benzopyrene by using 1,12-benzoperylene as internal standard. Muel and Lacroix[26] and Jäger[27] utilised a standard additions procedure for the determination of 3,4-benzopyrene in cigarette smoke, alcoholic drinks, water[26] and exhaust fumes;[27] these workers employed n-octane solvent at 83 or 77 K and measurement of the luminescence intensity at 403·0 nm with standard additions of 3,4-benzopyrene. Personov and Teplitskaya[28] and Florovskaya, Teplitskaya and Personov[29] have used the standard additions method for the determination via their quasi-linear luminescence emission of 3,4-benzopyrene, 1,12-benzoperylene and perylene in rocks and minerals of different origin.

Khesina and co-workers,[30,31] in methods that involve the use of both standard additions and internal standardisation, have described the determination of 9,10-dimethyl-1,2-benzanthracene, 1,2-benzanthracene, 1,2,5,6-dibenzanthracene, pyrene, 3,4-benzopyrene, perylene and 1,12-benzoperylene. Dikun, Krasnistkaya, Gorelova and Kalinina[32] have compared the standard additions, internal standard and combined methods for the determination of 3,4-benzopyrene, using its quasi-linear luminescence emission at low temperature. In the course of the investigations of the determination of various PAH compounds described above,

qualitative and quantitative methods for their determination in smoked fish,[32] diesel engine exhausts[33] and industrial oils[34] have been established.

Several possible difficulties that might hinder utilisation of the Shpol'skii effect for trace analysis have been reported, the first of which is the necessity to choose a suitable alkane solvent in order to observe the effect. Owing to the limited number of such solvents available it may not be possible to stimulate quasi-linear luminescence emission for some PAH compounds; conversely, this would possibly be advantageous if other compounds are to be determined in the presence of these non-emitting species. Shpol'skii, Klimova, Nersesova and Glyadkovskii[35] and Bolotnikova and Naumova[36] have investigated the influence of molecular aggregation and energy transfer on the intensity of quasi-linear luminescence emission. At low concentrations the spectra may be obscured owing to band emission from molecules aggregated inhomogeneously in the sample and not present in the crystalline matrix, while at high concentrations molecular aggregates excluded from the solvent lattice and which exhibit strong absorption but only weak emission may be formed. These effects might give rise to a restricted concentration range over which the luminescence emission exhibits a linear dependence on solute concentration, and to non-reproducible intensity from sample to sample, *i.e.*, poor precision. In addition, Dokunikhin, Kizel, Sapozhekov and Soloda[37] have observed that both the intensity and width of quasi-linear emission lines is dependent on the rate of freezing of sample solutions. In view of these considerations, it is surprising that the relatively large number of reports of the successful application of measurement of quasi-linear luminescence emission to real samples outlined above have appeared. For this reason, and in order to evaluate the potential of an apparently powerful selective technique of analysis, we have studied the Shpol'skii effect for a series of twenty-three PAH compounds in several n-alkane solvents. The technique is similar in its operation to conventional luminescence spectrometry, except for the requirement of a monochromator with moderately high resolution, and direct experiments can be undertaken in order to investigate its potential for qualitative and quantitative analysis.

Experimental

Apparatus—

A double monochromator spectrofluorimeter (American Instrument Co., Maryland) fitted with a potted RCA 1P 28 photomultiplier tube, xenon arc lamp continuum source and low-temperature sample cell accessory was employed so as to obtain preliminary spectral data at low resolution, thus facilitating the choice of excitation wavelength for higher-resolution studies in which a mercury-vapour discharge lamp source was used. Spectra were displayed on a Bryans X-Y recorder (Model 21000).

The quasi-linear luminescence emission of the compounds studied was recorded using the apparatus of higher resolution. Radiation from a medium pressure mercury-vapour discharge lamp (Wotan Hg/3) was focused into a light-tight sample cell compartment by using two silica lenses of 45 mm diameter and 75 and 50-mm focal length. Interference filters of narrow band width (50 × 50 mm, with a half-band width of 14 nm at 250 nm and 30 nm at 300 nm) were inserted between the source and sample-cell compartment for selection of the excitation wavelength. The sample tubes employed were constructed from silica tubing (Spectrosil) and were 200 mm in length, of 3 mm i.d. and 1-mm wall thickness and were sealed at one end. These tubes were used with the silica Dewar flask from the low-temperature accessory of the spectrofluorimeter used for the low-resolution studies. The liquid samples were plunged into liquid nitrogen contained in the Dewar flask so as to achieve rapid freezing, and the flask was then placed in the sample cell compartment so that the incident radiation was slightly defocused at the surface of the frozen sample.

This defocusing of the incident radiation was found to give rise to more reproducible signal intensities than when the source radiation was brought to a focus at the sample surface. A coil of Nichrome wire was positioned within the sample-cell compartment so as to be adjacent to the outer wall of the Dewar flask when the latter was placed in position. This wire was heated by passing a low a.c. current through it in order to minimise frosting of that part of the Dewar flask surface which is irradiated by the source and viewed by the detection system. A scanning grating monochromator (Optica, Model CF4) with a reciprocal linear

dispersion at the exit slit of 1·6 nm mm^{-1} was positioned so that its optical axis was at 90° to that of the source and sample cell.

Luminescence from the sample cell was focused on to the entrance slit of the monochromator by using a composite biconvex silica lens (40 mm in diameter and of 35-mm focal length). An end-window photomultiplier tube (EMI 9601B) was attached at the exit slit of the monochromator and operated at 1200 V by using a Brandenburg EHT supply. The luminescence signal was recorded directly at a potentiometric chart recorder (Servoscribe, Model RE 511.20) although for some experiments a microammeter (RCA, Model WV-84C) was employed for signal registration.

Reagents—

The n-alkane solvents used were n-pentane, n-hexane, n-octane and n-decane. These solvents were of laboratory-reagent grade and were used without further purification. Cyclohexane (laboratory-reagent grade) was purified by percolation through silica gel (60 to 120 mesh), which had been activated overnight at 120 to 130 °C. EPA solvent [diethyl ether - isopentane - ethanol (5 + 5 + 2)] was prepared with ether that had been dried with sodium wire, isopentane dried with sodium wire and percolated through silica gel and ethanol treated with potassium hydroxide and redistilled. Tetrahydrofuran was treated with potassium hydroxide and distilled. Other solvents for study of the matrix were used without pre-treatment.

Materials—

Samples of pure polynuclear aromatic hydrocarbon compounds were kindly donated by Tobacco Research Council Laboratories, Harrogate, British American Tobacco Co., Southampton, and Shell Research Ltd., Thornton Research Centre, Chester. 4,9-Di-t-butylpyrene and 3,5,8,10-tetraisopropylpyrene were kindly provided by Professor Arne Berg, University of Aarhus, Denmark.

Procedure—

Low-resolution excitation and emission spectra were recorded in EPA solvent at 77 K with the Aminco spectrofluorimeter with the low-temperature cell attachment or at room temperature with a silica sample cell (10 × 10 × 30 mm). For examination of the quasi-linear luminescence spectra stock solutions of the PAH compounds were prepared in cyclohexane; these solutions were diluted to 20 or 2 μg ml^{-1} concentration with the appropriate n-alkane and cyclohexane so that the final solutions examined contained 10 per cent. V/V of cyclohexane. These solutions were transferred into the silica sample tubes, which were then introduced directly into the Dewar flask cell that contained liquid nitrogen. Rapid freezing of the sample solutions was thus obtained. After the initial vigorous boiling action of the liquid nitrogen had subsided, the luminescence emission spectrum was scanned (using a pre-selected excitation wavelength) at 6 nm min^{-1}.

Results

Spectral characteristics—

Cyclohexane is a generally suitable solvent for PAH compounds and its presence in concentrations up to 10 per cent. V/V in the n-alkane solvents used for low-temperature luminescence work has been demonstrated not to disturb appreciably the spectra obtained.[17,22,26] As only small amounts of some of the PAH compounds examined were available, it was therefore decided to use cyclohexane for their dissolution and n-alkane - cyclohexane mixtures (90+10) for dilution of the stock PAH solutions for the examination of their luminescence spectra. For some PAH compounds quasi-linear luminescence emission is observed only at 77 K when an alkane solvent of matching molecular dimensions is employed. Thus, in a survey of the occurrence of the Shpol'skii effect for a range of twenty-two PAH compounds, it has been necessary to employ several n-alkane solvents in order to choose that which was the most suitable for each compound.

An examination of earlier work[26,30,31,34] revealed that n-octane had proved to be suitable as a solvent for study of the Shpol'skii effect for some PAH compounds. Initially, therefore, the low-temperature luminescence emission spectra of all twenty-two compounds were

recorded in n-octane - cyclohexane (90+10). Excitation wavelengths were selected from the corresponding low-temperature excitation spectra that were obtained by using the low-resolution double-monochromator spectrofluorimeter. The wavelength was chosen so as to correspond to that of the most intense low-wavelength excitation peak in order to minimise observed scattered light during the study of the emission spectrum. Of the compounds examined in n-octane - cyclohexane solvent at 77 K, triphenylene, chrysene, perylene, pyrene, 3,5,8,10-tetraisopropylpyrene, 3,4-benzopyrene, 1,2-benzopyrene, 1,2,3,4-dibenzopyrene, 1,2,4,5-dibenzopyrene, 3,4,9,10-dibenzopyrene, 1,2-benzanthracene and 1,2,5,6-dibenzanthracene exhibit quasi-linear luminescence in which emission peak half-widths of 0·5 nm or less were recorded.*

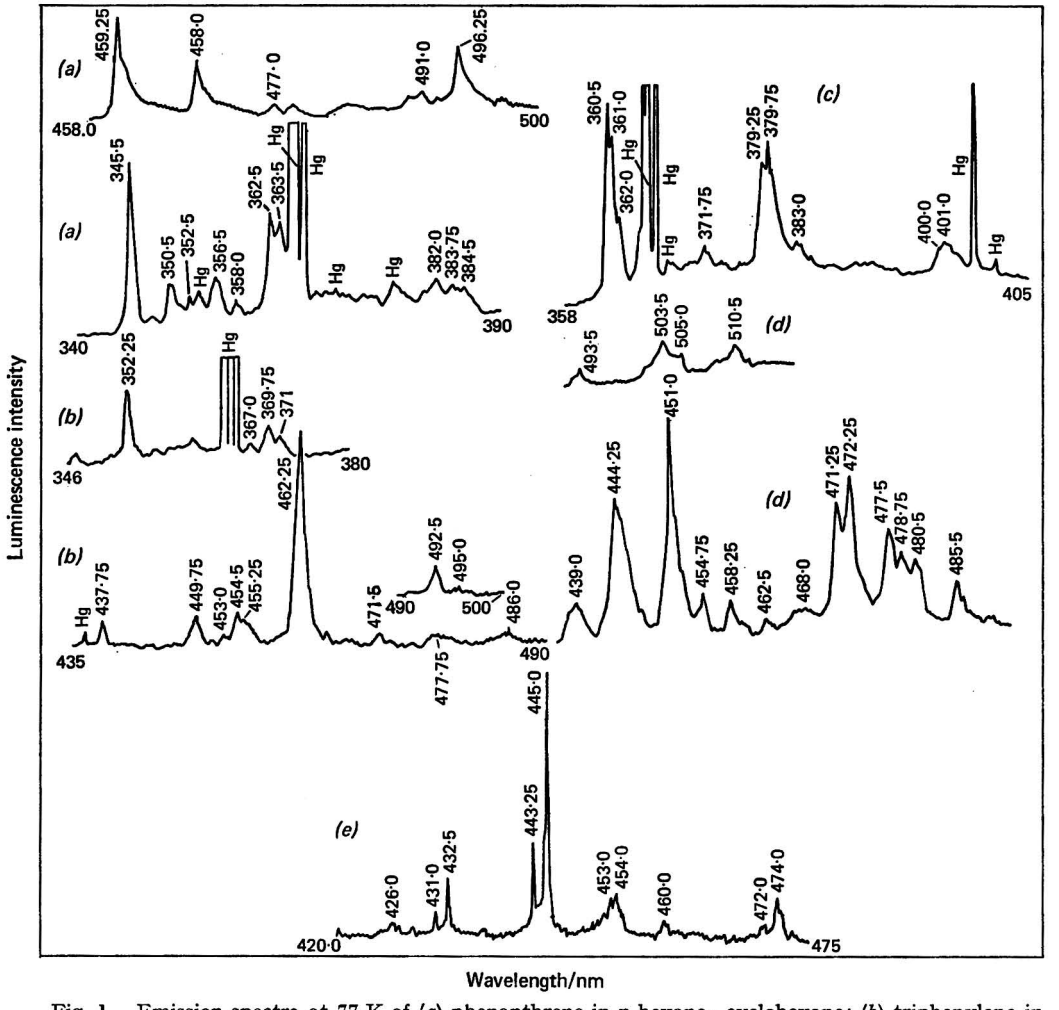

Fig. 1. Emission spectra at 77 K of (*a*) phenanthrene in n-hexane - cyclohexane; (*b*) triphenylene in n-octane - cyclohexane; (*c*) chrysene in n-octane - cyclohexane; (*d*) perylene in n-octane - cyclohexane; and (*e*) coronene in n-hexane

The spectra observed for 2 or 20 μg ml^{-1} solutions of these compounds in n-octane - cyclohexane at 77 K are shown in Figs. 1 to 5. The compounds 3,4,8,9-dibenzopyrene (Fig. 6), indeno[1,2,3-*cd*]pyrene, benzo[*a*]naphtho[8,1,2-*cde*]naphthacene (Fig. 7), 3-methylcholanthrene, 7,12-dimethyl-1,2-benzanthracene (Fig. 3), 3-methylpyrene, 4,9-di-t-butylpyrene

*The nomenclature used follows that of reference 1.

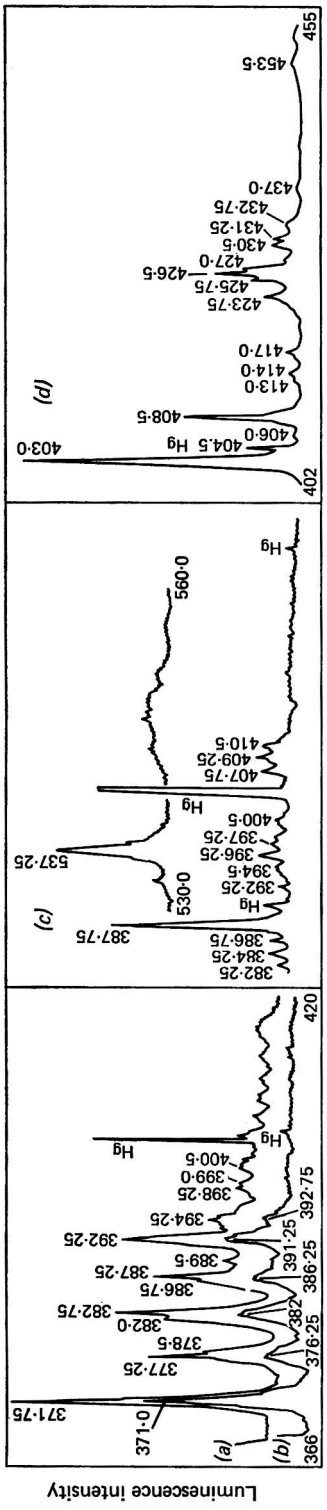

Fig. 2. Emission spectra at 77 K of (*a*) pyrene in n-octane - cyclohexane; (*b*) pyrene in EPA; (*c*) 1,2-benzopyrene in n-octane - cyclohexane; and (*d*) 3,4-benzopyrene in n-octane - cyclohexane

(Fig. 5), phenanthrene (Fig. 1) and 9,10-dimethylanthracene (Fig. 3) exhibited intense but broad-band luminescence emission in n-octane - cyclohexane solvent. In these spectra, typical peak half-widths greater than 1·0 nm were observed. Anthracene exhibited a very broad spectrum (Fig. 6). Some of these compounds (phenanthrene, anthracene and 9,10-dimethylanthracene) were examined in n-hexane - cyclohexane solvent and gave somewhat narrower half-band widths in their luminescence spectra (Figs. 1, 3 and 6). 3,4,8,9-Dibenzopyrene was examined also in n-decane - cyclohexane (90 + 10) and gave a more well defined spectrum than that obtained in n-octane - cyclohexane (Fig. 6). A schematic presentation of the luminescence characteristics of fifteen of these compounds appears in Fig. 8.

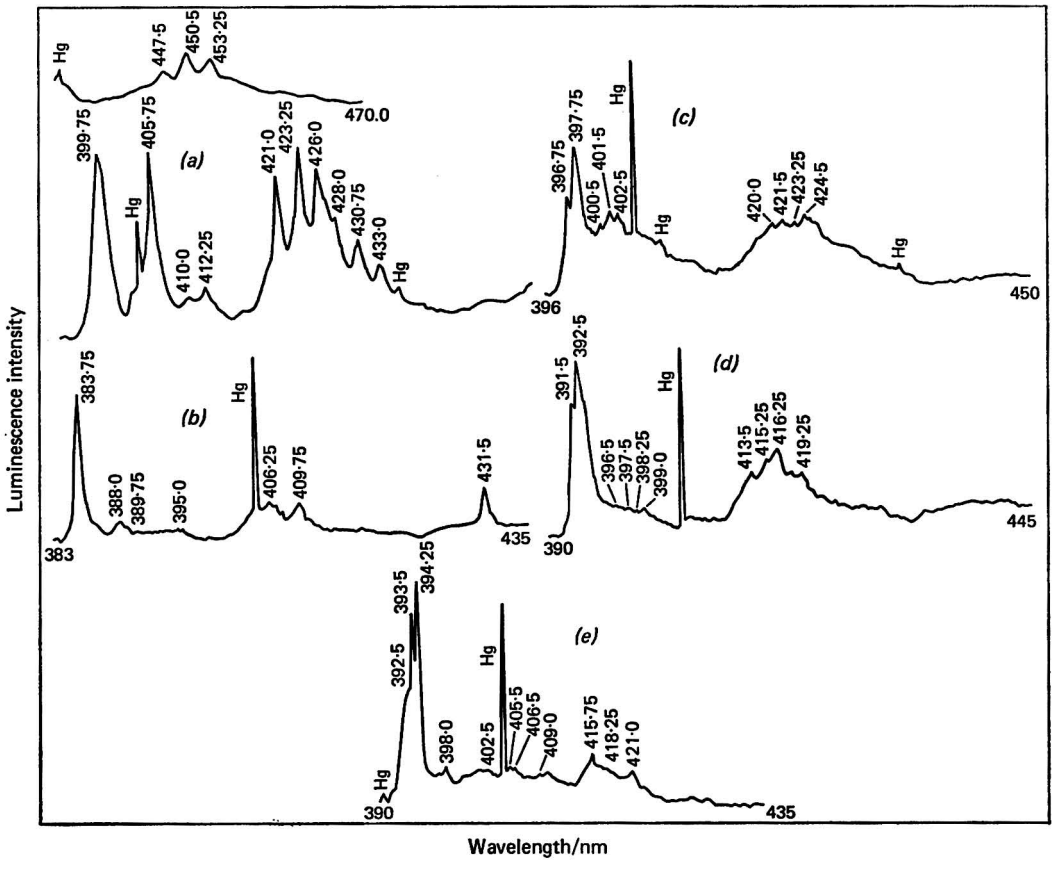

Fig. 3. Emission spectra at 77 K of (*a*) 9,10-dimethylanthracene in n-hexane - cyclohexane; and (*b*) 1,2-benzanthracene, (*c*) 7,12-dimethyl-1,2-benzanthracene, (*d*) 3-methylcholanthrene and (*e*) 1,2,5,6-dibenzanthracene in n-octane - cyclohexane

Most early observations of the Shpol'skii effect for PAH compounds were made utilising n-alkane solvents and it has generally been accepted that matrices formed from the straight-chain hydrocarbon solvents of suitable molecular dimensions are required in order to obtain quasi-linear emission spectra. A striking demonstration of the need to select the correct n-alkane solvent can be made by comparison of the spectrum obtained for anthracene in n-hexane - cyclohexane (Fig. 6), in which quasi-linear emission is obtained, with the spectrum for anthracene in n-octane - cyclohexane (Fig. 6), in which only a very broad emission is observed. The restriction of the effect to relatively non-polar solvents, however, would result in limited analytical utility and restrict the study of the technique to those compounds which are soluble in these solvents.

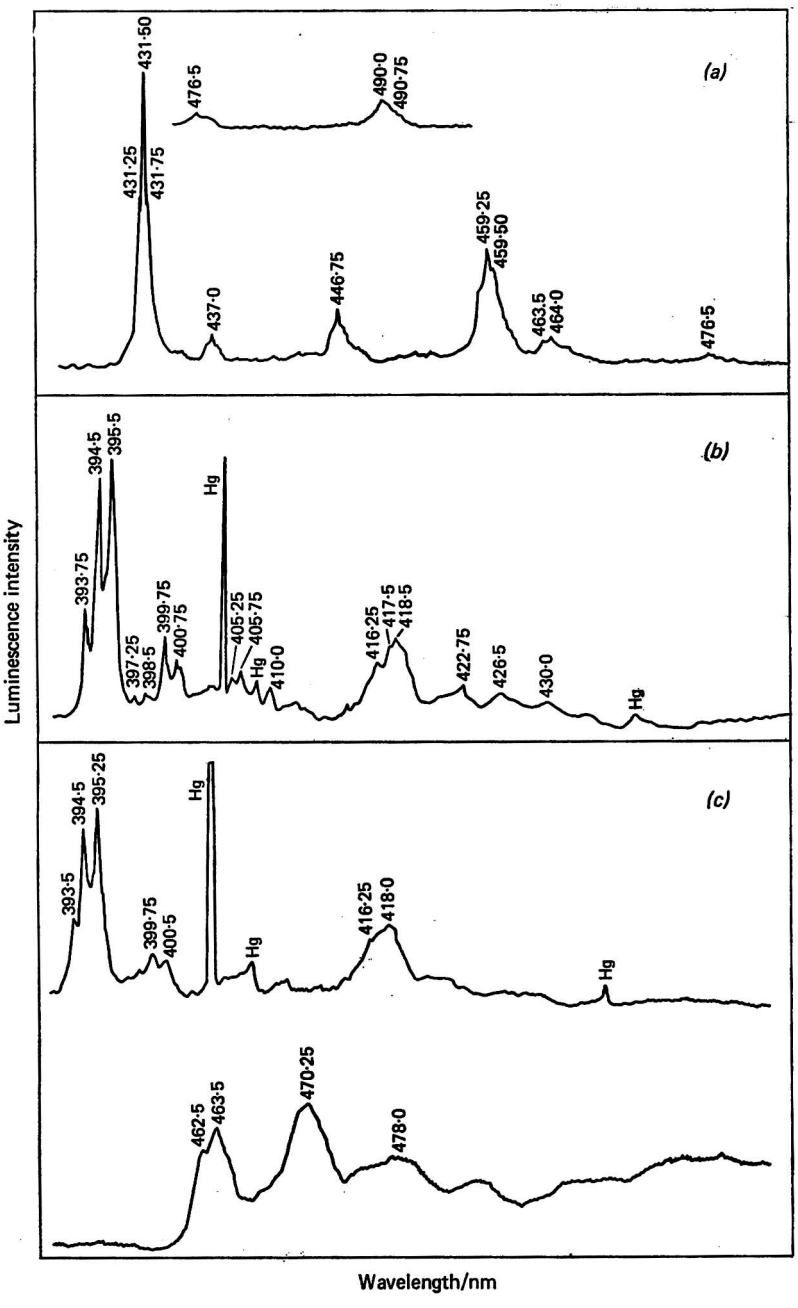

Fig. 4. Emission spectra at 77 K of some dibenzopyrenes in n-octane - cyclohexane: (*a*) 3,4,9,10- dibenzopyrene; (*b*) 1,2,4,5-dibenzopyrene; and (*c*) 1,2,3,4-dibenzopyrene

A preliminary qualitative study of the use of other solvents was therefore undertaken. The model PAH compound chosen for study was coronene; this hydrocarbon exhibits a simple and well defined quasi-linear emission spectrum in n-hexane (Fig. 1). The emission spectra of solutions containing 20 μg ml^{-1} of coronene in dioxan, pentanol, carbon tetrachloride, chloro-

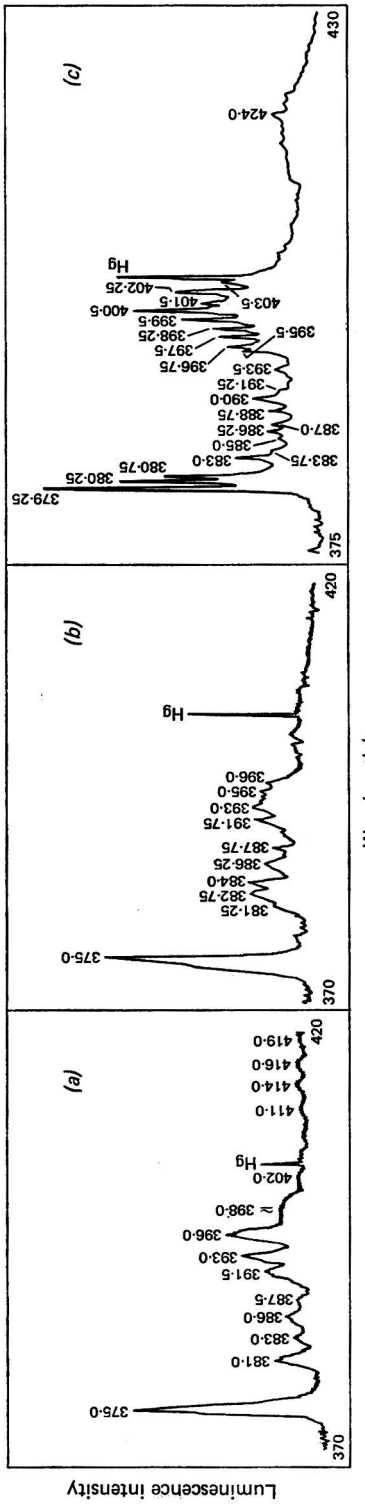

Fig. 5. Emission spectra at 77 K of some alkylpyrenes in n-octane - cyclohexane: (*a*) 3-methylpyrene; (*b*) 4,9-di-t-butylpyrene; and (*c*) 3,5,8,10-tetraisopropylpyrene

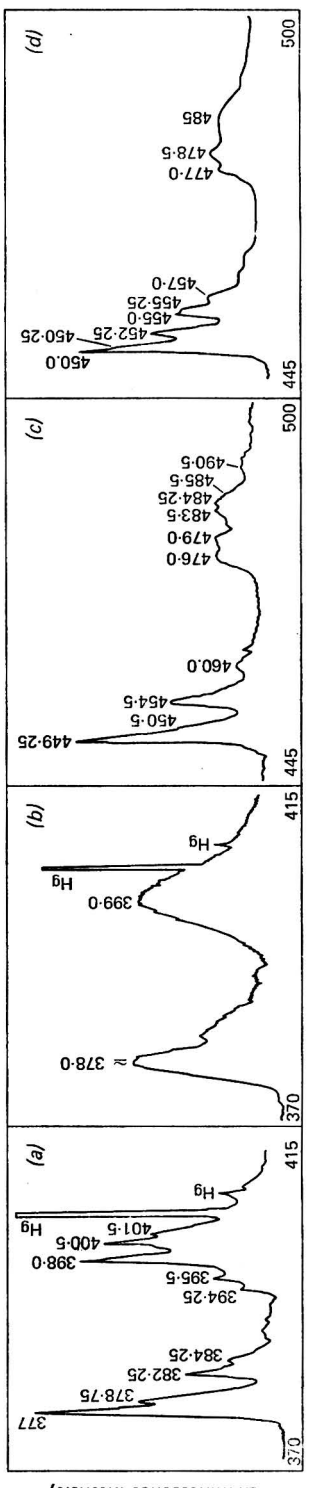

Fig. 6. Solvent effect observed in the emission spectra at 77 K for anthracene with (*a*) n-hexane - cyclohexane solvent and (*b*) n-octane - cyclohexane solvent; and for 3,4,8,9-dibenzopyrene with (*c*) n-octane - cyclohexane solvent and (*d*) n-decane - cyclohexane solvent

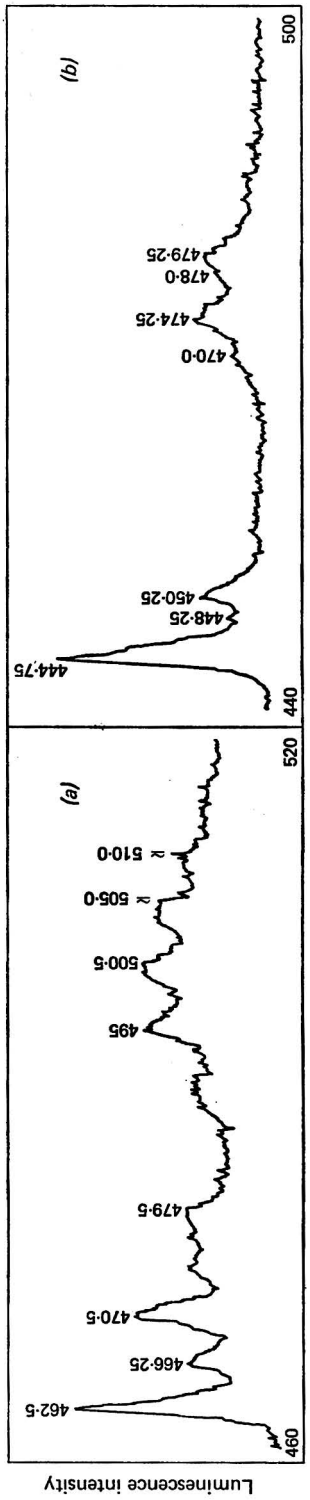

Fig. 7. Emission spectra at 77 K in n-octane - cyclohexane of (*a*) indeno[1,2,3-*cd*]pyrene; and (*b*) benzo[*a*]naphtho[8,1,2-*cde*]naphthacene

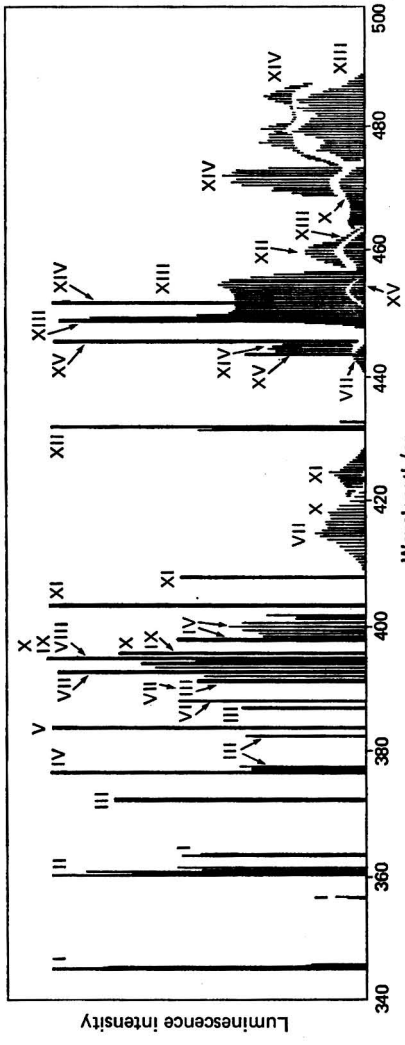

Fig. 8. Schematic presentation of the emission spectra at 77 K of some of the PAH compounds studied: (I) phenanthrene and (IV) anthracene in n-hexane - cyclohexane; (II) chrysene, (III) pyrene, (V) 1,2-benzanthracene, (VI) 1,2-benzopyrene, (VII) 3-methylcholanthrene, (VIII) 1,2,5,6-dibenzanthracene, (IX) 1,2,4,5-dibenzopyrene, (X) 1,2,3,4-dibenzopyrene, (XI) 3,4-benzopyrene, (XII) 3,4,9,10-dibenzopyrene, (XIII) 3,4,8,9-dibenzopyrene and (XIV) perylene in n-octane - cyclohexane; and (XV) coronene in n-hexane

form, bromoform, diethyl ether, dimethylformamide, 1,1,2,2-tetrachloroethane and tetrahydrofuran were examined at 77 K. Only with tetrahydrofuran was a well defined quasi-linear emission spectrum observed for coronene [Fig. 9 (*a*)]. The corresponding phosphorescence emission spectrum for coronene is shown in Fig. 10. Of twenty-three PAH compounds studied, quasi-linear emission was also observed for 4,9-di-t-butylpyrene, 1,2-benzopyrene and 1,2,5,6-dibenzanthracene in tetrahydrofuran. The spectra observed for these compounds are shown in Figs. 9 and 11 and the data recorded are listed in Table I. Although spectral emission band widths that were greater than expected for the Shpol'skii effect were observed for 3-methylcholanthrene, perylene and 3,4,9,10-dibenzopyrene in tetrahydrofuran as shown in Figs. 9 and 11, sharp and useful spectra were obtained for these compounds.

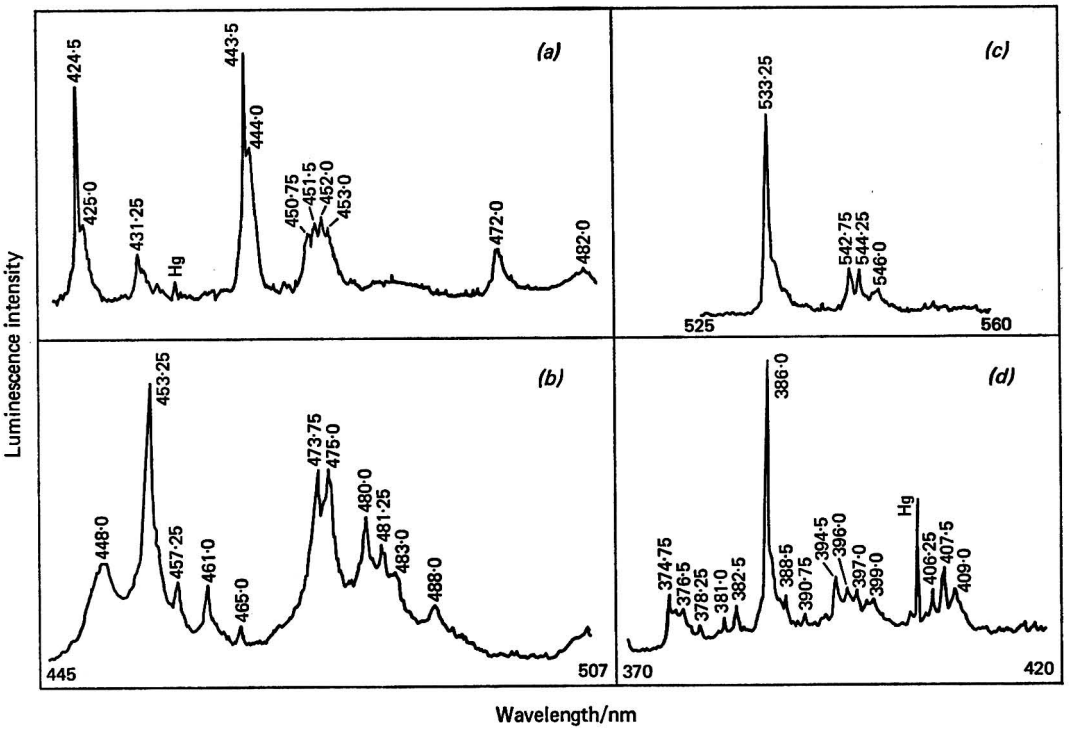

Fig. 9. Emission spectra at 77 K of some PAH compounds in tetrahydrofuran: (*a*) coronene; (*b*) perylene; (*c*) 1,2-benzopyrene (phosphorescence spectrum); and (*d*) 1,2-benzopyrene (fluorescence spectrum)

Good agreement is observed in the wavelength assignments made for the principal quasi-linear luminescence emission maxima in this work with those recorded earlier for several of the compounds studied by other workers.[26,38-42] The wavelength reproducibility of these emission maxima and the relative freedom from overlap of the narrow-band "quasi-line" spectra compared with that obtained in solution at room temperature or in glass-forming organic solvents such as EPA at 77 K suggest that the quasi-linear spectra may be extremely useful for qualitative identification of PAH compounds. Fig. 2 shows the luminescence emission spectrum at 77 K for pyrene in EPA glass and the corresponding quasi-linear emission spectrum at 77 K in n-octane - cyclohexane. Both spectra were recorded with the high-resolution instrumentation. The gain in structure obtained by utilising the Shpol'skii effect and the possibility of less ambiguous identification of this compound in the presence of others is clearly seen.

In order to demonstrate the "fingerprinting" ability of the technique, a synthetic mixture of eight PAH compounds was prepared and its low-temperature luminescence emission spectrum was recorded at 77 K in n-octane - cyclohexane solvent; Fig. 12 shows the

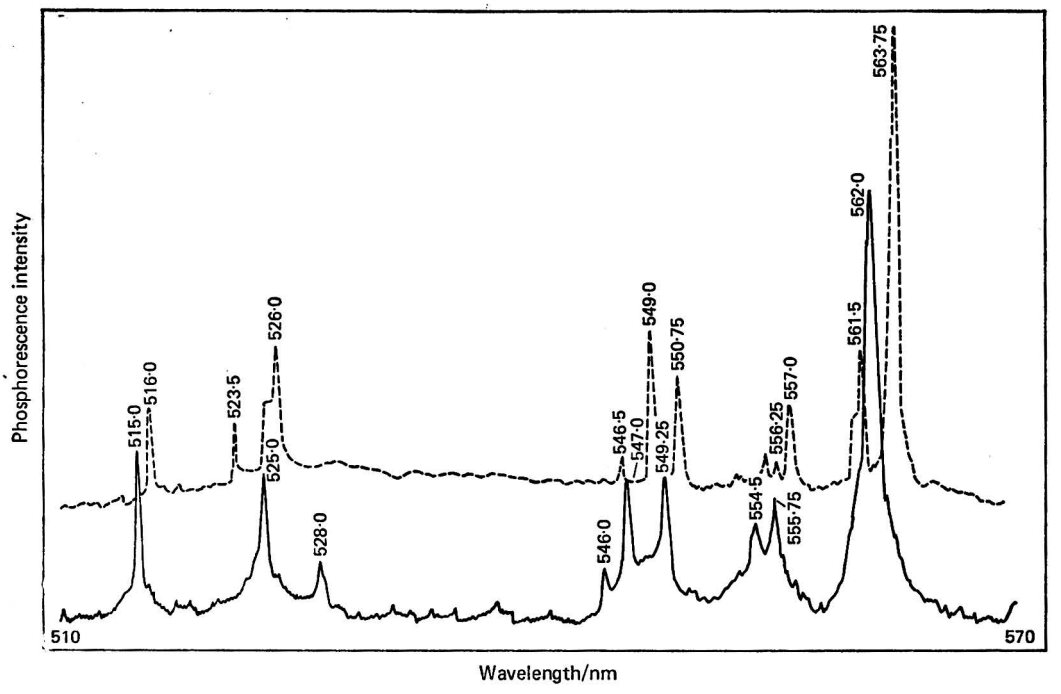

Fig. 10. Phosphorescence emission spectra of coronene in n-hexane (broken line) and in tetrahydrofuran (solid line)

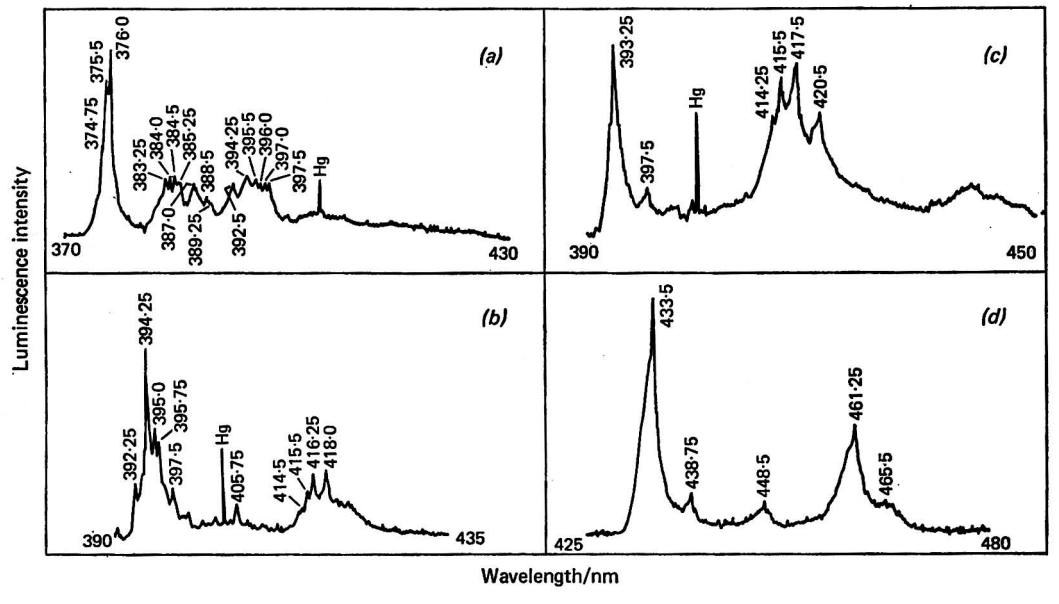

Fig. 11. Emission spectra at 77 K of some PAH compounds in tetrahydrofuran: (*a*) 4,9-di-t-butylpyrene; (*b*) 1,2,5,6-dibenzanthracene; (*c*) 3-methylcholanthrene; and (*d*) 3,4,9,10-dibenzopyrene

emission spectrum obtained. Each of the eight hydrocarbons present in the mixture is readily identified from the principal luminescence emission maxima observed. Even when some overlap occurs for certain principal peaks, there is sufficient information present in the minor features of the spectrum of each compound to permit its detection by using alternative peak wavelengths.

TABLE I

EMISSION AT 77K OF SOME PAH COMPOUNDS IN TETRAHYDROFURAN

Excitation wavelength 300 nm

Compound	Wavelengths of principal emission maxima
Coronene	424·5 (s), 425·0 (m), 431·25 (w), *443·5* (vs), 444·0 (s), 450·75 (m), 451·5 (m), 452·0 (m), 453·0 (m), 472·0 (m), 482·0 (w) p: 515·0 (m), 525·0 (m), 528·0 (w), 546·0 (w), 547·0 (m), 549·25 (m), 554·5 (m), 555·75 (m), *562·0* (vs)
Perylene	448·0 (m), *453·25* (vs), 457·25 (m), 461·0 (m), 465·0 (w), 473·75 (s), 475·0 (s), 480·0 (m), 481·25 (m), 483·0 (m), 488·0 (w)
1,2-Benzopyrene ..	374·75 (m), 376·50 (m), 378·25 (w), 381·0 (w), 382·5 (m), *386·0* (vs), 388·5 (m), 390·75 (w), 394·5 (m), 396·0 (m), 397·0 (m), 399·0 (m), 406·25 (m), 407·5 (m), 409·0 (m) p: *533·25* (s), 542·75 (m), 544·25 (m), 546·0 (w)
3,4,9,10-Dibenzopyrene	*433·5* (vs), 438·75 (w), 448·5 (w), 461·25 (m), 465·5 (d,w)
3-Methylcholanthrene ..	*393·25* (vs), 397·5 (w), 414·25 (m), 415·5 (m), 417·5 (m), 420·5 (m)
1,2,5,6-Dibenzanthracene	392·25 (m), *394·25* (vs), 395·0 (m), 397·5 (w), 405·75 (w), 414·5 (vw), 415·5 (w), 416·25 (m), 418·0 (m).
4,9-Di-t-butylpyrene ..	374·75 (m), 375·5 (s), *376·0* (vs), 383·25 (m), 384·0 (m), 384·5 (m), 385·25 (m), 387·0 (m), 388·5 (m), 389·25 (w), 392·5 (m), 394·25 (m), 395·5 (m), 396·0 (m), 397·0 (m), 397·5 (m)

Concentration of all compounds in tetrahydrofuran: 20 μg ml^{-1}.
vs, very strong emission; s, strong; w, weak; m, medium; d, diffuse; p, phosphorescence emission.
Wavelengths in italics indicate the most intense peaks.

The detection limits given for the compounds studied can be defined as that concentration of hydrocarbon in the solvent employed which gives a signal to noise ratio of 2 at the wavelength of the principal quasi-linear emission. The wavelengths (nm) used and the values (μg ml^{-1}) obtained for these compounds were: phenanthrene (345·5, 0·1); triphenylene (462·25, 0·1); chrysene (360·5, 0·05); perylene (451·0, 0·05); pyrene (371·75, 0·05); 3·4-benzopyrene (403·0, 0·005); 1,2-benzopyrene (387·75, 0·07); 1,2,3,4-dibenzopyrene (395·25, 0·08); 1,2,4,5-dibenzopyrene (395·5, 0·08); 3,4,8,9-dibenzopyrene (449·25, 0·04); 3,4,9,10-dibenzopyrene (431·5, 0·1); anthracene (377·0, 0·05); 1,2-benzanthracene (383·75, 0·1); 7,12-dimethyl-1,2-benzanthracene (397·75, 0·2); 3-methylcholanthrene (392·5, 0·2); 1,2,5,6-dibenzanthracene (394·25, 0·1); 9,10-dimethylanthracene (405·75, 0·05); 3-methylpyrene (375·0, 0·2); 4,9-di-t-butylpyrene (375·0, 0·2); 3,5,8,10-tetraisopropylpyrene (379·25, 0·1); indeno[1,2,3-*cd*]pyrene (462·5, 0·3); benzo[*a*]naphtho[8,1,2-*cde*]naphthacene (444·75, 0·3); and coronene (445·0, 0·1).

These limits are estimates only and would be subject to considerable improvement if a more efficient optical arrangement for collection of the emitted radiation and more sophisticated detector electronics were used.

QUANTITATIVE STUDIES—

The variation in intensity of luminescence emission at 77 K with concentration was examined for the four dibenzopyrene compounds available to us in n-octane - cyclohexane solvent, and the intensity of the quasi-linear emission for 3,4,9,10-dibenzopyrene, 1,2,4,5-dibenzopyrene and 1,2,3,4-dibenzopyrene was measured at 431·50, 395·50 and 395·25 nm, respectively. This variation was also examined for 1,2,3,4-dibenzopyrene at its broad emission peak at 470·25 nm. Measurement at this wavelength, rather than at 395·25 nm, would be advantageous in the determination of this compound in the presence of 1,2,4,5-dibenzopyrene as it avoids interference from the emission of the latter compound at 395·5 nm. The luminescence growth curves obtained are plotted logarithmically in Fig. 13.

For the compounds examined, the quasi-linear emission intensity is linear over a concentration range of two orders of magnitude. Fig. 13 also shows that similar intensity, slope and range of linearity are obtained at both 395·25 and 470·25 nm for the luminescence growth

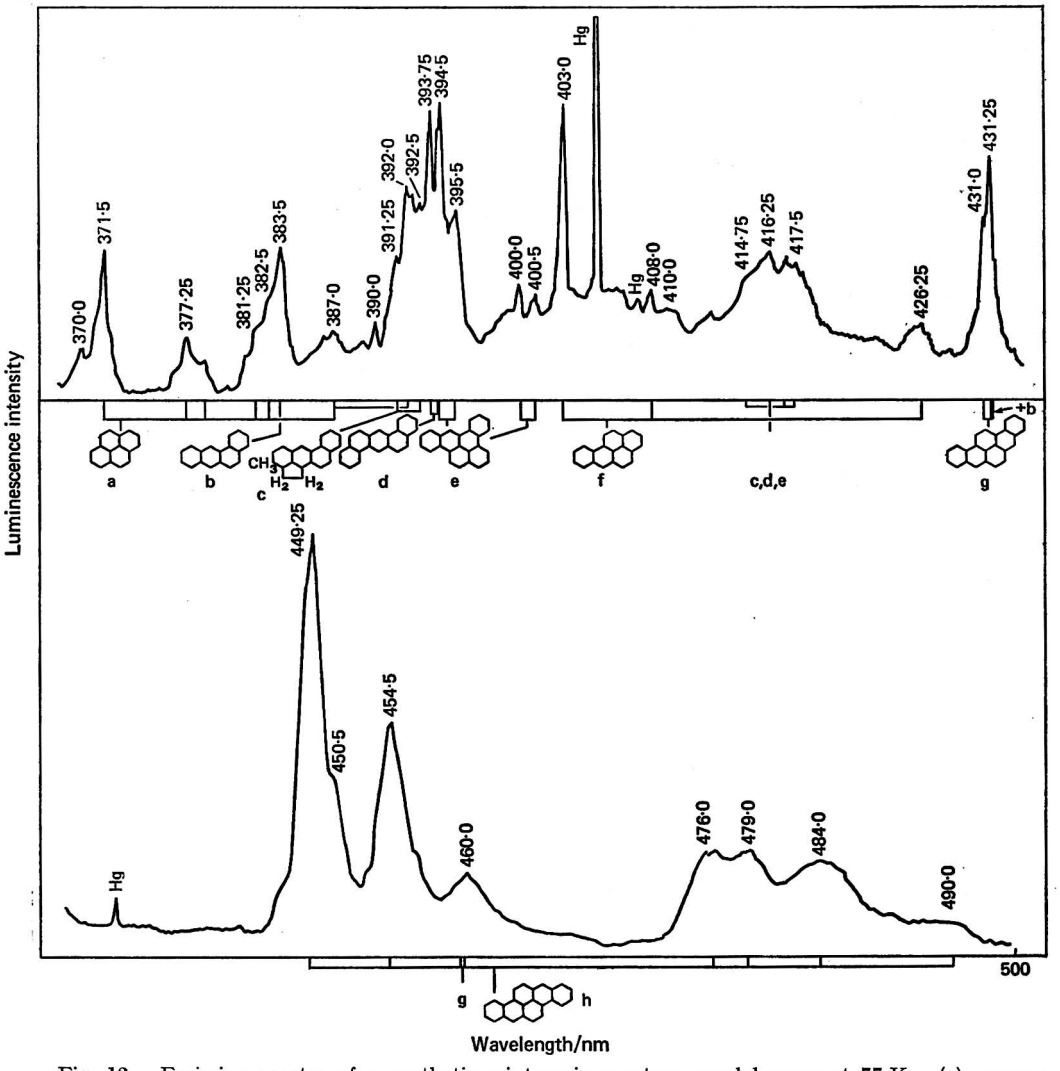

Fig. 12. Emission spectra of a synthetic mixture in n-octane - cyclohexane at 77 K: (*a*) pyrene; (*b*) 1,2-benzanthracene; (*c*) 3-methylcholanthrene; (*d*) 1,2,5,6-dibenzanthracene; (*e*) 1,2,4,5-dibenzopyrene; (*f*) 3,4-benzopyrene; (*g*) 3,4,9,10-dibenzopyrene; and (*h*) 3,4,8,9-dibenzopyrene

curve of 1,2,3,4-dibenzopyrene. Although 3,4,8,9-dibenzopyrene does not show quasi-linear luminescence emission in n-octane - cyclohexane solvent at 77 K it can be seen in Fig. 13 that a similar intensity and range of linearity is observed for the luminescence of this compound when measured at the wavelength of its more intense emission at 449·25 nm.

Although it appears that direct measurement of the intensity of quasi-linear luminescence emission is capable of permitting the direct quantitative determination of compounds such as the dibenzopyrenes, in the examination of real samples several difficulties exist that lead to the requirement for the use of a standard additions procedure and the use of an internal standard. Thus when other compounds are present with the PAH compound to be determined, their absorption spectra may overlap that of the analyte molecule and give rise to an "inner filter" effect and low fluorescence intensities; energy transfer between the analyte molecule and others present in the sample may also lead to inaccurate values of intensity of quasi-linear emission compared with those expected in the absence of other compounds; the

use of the standard additions method of analysis minimises these inaccuracies. Variation of experimental measurement conditions, such as rate of freezing or the reproducibility with which the sample cell can be placed in the optical path, can lead to poor precision in measurement of quasi-linear luminescence intensity. These effects can be minimised by the use of an internal standard in quantitative work.

A combined standard addition - internal standard procedure was adopted for quantitative determination of the dibenzopyrene compounds in n-octane - cyclohexane solvent. The internal standard employed was selected so that the wavelength of its quasi-linear luminescence emission did not overlap that of the quasi-linear emission of the compound to be determined. It is, of course, not possible to avoid such overlapping in the corresponding excitation spectra; it is necessary for radiation transmitted by the single filter used to excite luminescence from both analyte compound and internal standard, so that some overlap in excitation spectra must occur if the internal standard technique is to be used in this manner.

1,2,4,5-Dibenzopyrene, 3,4,9,10-dibenzopyrene and 3,4,8,9-dibenzopyrene were determined by measurement of their quasi-linear luminescence in n-octane - cyclohexane at 77 K at the wavelengths employed in order to construct their luminescence growth curves shown in Fig. 13. The quasi-linear emission of 3,4-benzopyrene at 403 nm was used as internal standard for the determination of 1,2,4,5-dibenzopyrene and 3,4,9,10-dibenzopyrene and a standard additions procedure was employed in order to produce the calibration graphs shown in Fig. 14 (*a*) and (*b*). For the determination of 3,4,8,9-dibenzopyrene in n-octane - cyclohexane the quasi-linear emission of 1,2-benzopyrene at 387·75 nm was used for internal standardisation; the calibration graph obtained is shown in Fig. 14 (*c*). In each instance the ratio of the observed intensities for the luminescence of the analyte and internal standard are plotted against concentration.

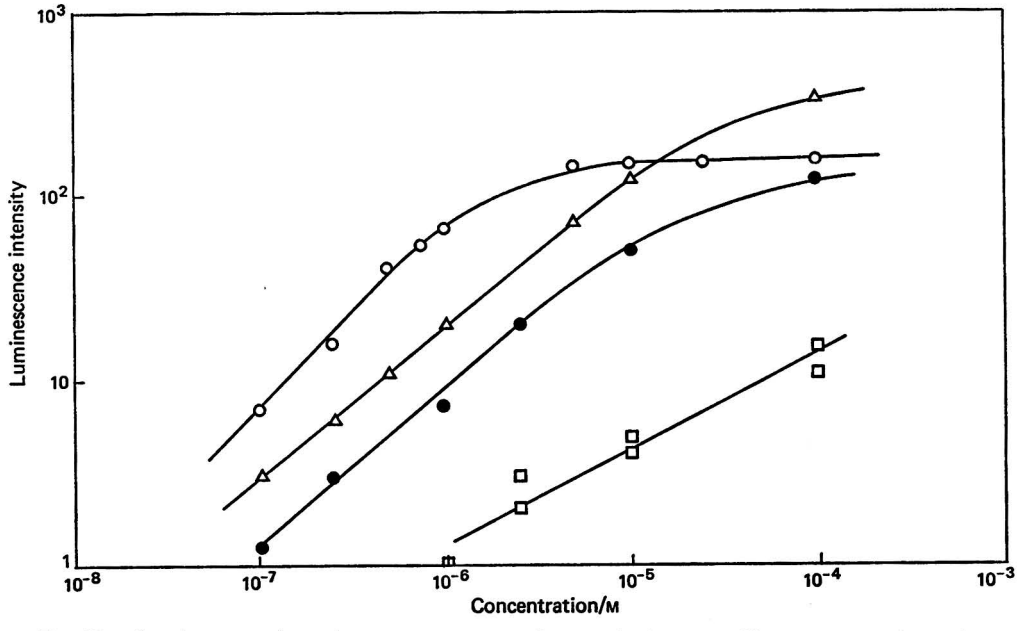

Fig. 13. Luminescence intensity *versus* concentration graphs for some dibenzopyrenes determined at 77 K in n-octane - cyclohexane: ○, 3,4,8,9-dibenzopyrene; △, 3,4,9,10-dibenzopyrene; ●, 1,2,4,5-dibenzopyrene; and □, 1,2,3,4-dibenzopyrene

Although the use of an internal standardisation procedure leads to improved precision by decreasing the effects of random errors in the measurement, an "inner filter" effect occurs in a manner similar to that mentioned above owing to the overlap of the excitation spectrum of the internal standard compound with that of the compound determined; lower quasi-linear luminescence emission intensities for the analyte compound are thus obtained in the presence

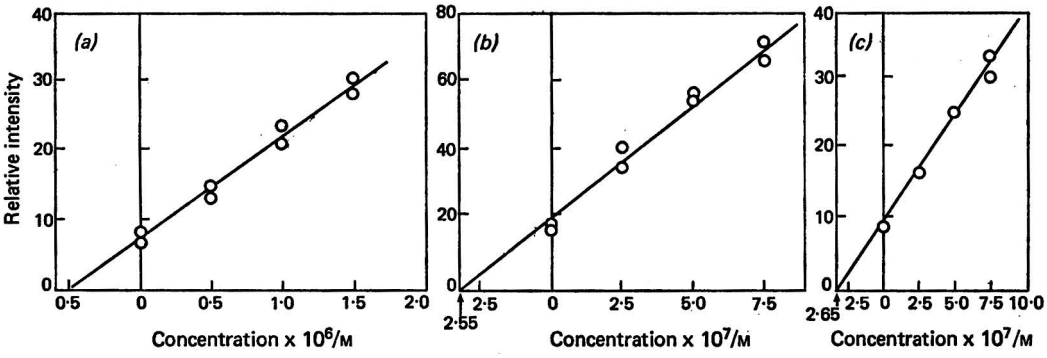

Fig. 14. Working curves for (*a*) 1,2,4,5-, (*b*) 3,4,9,10- and (*c*) 3,4,8,9-dibenzopyrene using the combined method (addition method and internal standard) at 77 K in n-octane - cyclohexane

of the internal standard compound. Improvement in precision is obtained over the analytical working range when the internal standardisation procedure is employed, but the decrease in luminescence signal intensity that results leads to some deterioration in detection limit for the compounds studied. Table II shows the effect of the presence of equimolar or greater concentrations of some other PAH compounds on the quasi-linear luminescence of the dibenzopyrene compounds excited at 300 nm in n-octane - cyclohexane. The percentage suppression or enhancement of the luminescence signal in the presence of these compounds is listed. In almost all instances suppression of quasi-linear emission of the dibenzopyrenes occurs owing to the "inner filter" effect or, possibly, by energy transfer. The enhancement of 1,2,3,4-dibenzopyrene emission at 395·5 nm in the presence of 1,2,4,5-dibenzopyrene is caused by the direct overlap of their quasi-linear emission at this wavelength; no effect is observed when the luminescence of 1,2,3,4-dibenzopyrene is measured at 470·5 nm.

Table II

Effect of other PAH compounds on measured luminescence intensities at 77K for dibenzopyrene compounds studied

Results are expressed as change in luminescence intensity, per cent.

	Interferent							
	1,2,3,4-DBP	1,2,4,5-DBP	3,4,8,9-DBP	3,4,9,10-DBP	3,4-BP	1,2-BP	1,2,5,6-DBA	PER[e]
1,2,3,4-Dibenzopyrene	—	+226[a]; Nil[b]	−35[a]; Nil[b]	−35[a]; Nil[b]	−54·0[a,b]	—	+22·2[a,g]; −29·6[b]	—
1,2,4,5-Dibenzopyrene	−34·5	—	−35·0	−57·2	−60·5	—	−22·9[f]	—
3,4,8,9-Dibenzopyrene	−37·8	−33·4	—	−14·9	−58·0	−45·5[c]	—	−22·8; −35·8[d]
3,4,9,10-Dibenzopyrene	−17·8	−31·5	−45·8	—	−20·6 −56·2[d]	—	−22·8 −32·8[d]	−31·5 −82·5[d]

[a] at 395·5 nm; [b] at 470·5 nm; [c] four-fold excess of 1,2-BP; [d] ten-fold excess; [e] perylene is not excited with the filter employed; [f] at ten-fold excess there is direct interference; [g] at ten-fold excess there is direct interference in the emission at 395·5 nm and suppression (−60 per cent.) at the 470·0 nm emission.

3,4-Benzopyrene, 1,2-benzopyrene, 1,2,5,6-dibenzanthracene and perylene appear as 3,4-BP, 1,2-BP, 1,2,5,6-DBA and PER, respectively, in the table; DBP denotes dibenzopyrene.

Conclusions

Our preliminary study reported here confirms that the high selectivity claimed for the identification of PAH compounds utilising the Shpol'skii effect at 77 K is readily attained. The quasi-linear emission spectra can be used for unambiguous "fingerprinting" of these compounds in solution at trace concentrations. The wavelength assignments for the principal quasi-linear luminescence emission maxima observed agree well with those made by other workers. At present, the application of this fingerprinting technique is limited to those

aromatic hydrocarbons which are soluble in n-alkanes. The ability to obtain quasi-linear emission spectra in tetrahydrofuran, however, suggests that the technique may be extended to compounds that are not soluble in these solvents and that are more polar than PAH compounds.

Although it is necessary to match the molecular dimensions of solute and solvent species, this requirement can be used to advantage in order to improve selectivity by choice of suitable solvent if spectral overlap at major luminescence peaks is observed for some compounds. The quantitative use of the Shpol'skii effect for trace analysis requires careful calibration and use of a combined internal standard - standard additions technique so as to minimise the effects of energy transfer, the inner filter effect and experimental variables and to attain acceptable precision and accuracy. It has been shown[42,43] that the corresponding absorption spectra of PAH compounds at 77 K may be quasi-linear in character. The use of a narrow-line excitation source, such as a tunable dye laser, would therefore provide even greater selectivity and sensitivity for the analytical application of the Shpol'skii effect.

One of us (C.G. de L.) thanks the University of Brasilia for study leave and UNESCO for the grant of a Fellowship. We also thank those agencies mentioned in the text for the provision of samples for examination.

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Received *October* 23*rd*, 1973
Accepted *January* 2*nd*, 1974

Analyst, June, 1974, Vol. 99, *pp.* 355–359

Application of the Spectrophotometric Determination of Nickel and Cobalt in Mixtures With Bipyridylglyoxal Dithiosemicarbazone to the Analysis of Catalysts

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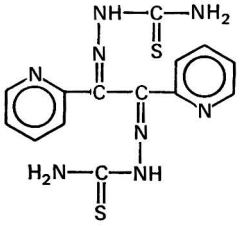
Bipyridylglyoxal dithiosemicarbazone forms a complex with nickel(II) at pH 5·2, which can be extracted into chloroform ($\lambda_{max.}$ = 410 nm). A similar complex is obtained with cobalt(II), but is not extractable in this solvent, thus allowing nickel and cobalt to be determined in mixtures. Two procedures are proposed for the accurate analysis of such mixtures in which 1 p.p.m. of one of the ions can be determined accurately in the presence of as much as 5 p.p.m. of the other. One of the procedures has been applied to the determination of nickel and cobalt in industrial catalysts and the results obtained have been compared with those obtained by atomic-absorption spectrophotometry. Satisfactory results were obtained.

The work described in this paper forms part of an investigation into the use of dithiosemicarbazones as analytical reagents. In a previous paper[1] we have studied the reactions between iron(II) and (III) ions and bipyridylglyoxal dithiosemicarbazone (BGT)—



In this paper the determination of nickel(II) and cobalt(II), both separately and in mixtures, with the above reagent and its application to the analysis of catalysts are described.

Experimental

Apparatus—

Spectrophotometers—Unicam SP800 and SP600 spectrophotometers, equipped with 1·0-cm quartz or glass cells, were used for ultraviolet and visible-light absorbance measurements. A Perkin-Elmer 402 atomic-absorption spectrophotometer was also used.

Digital pH meter—A Philips PW 9408 instrument, with glass - calomel electrodes, was used.

Solutions—

All solvents and reagents were of analytical-reagent grade.

Bipyridylglyoxal dithiosemicarbazone solution—A 0·1 per cent. *m/V* solution in ethanol. The reagent is synthesised from bipyridylglyoxal and thiosemicarbazide.[2]

Standardised solutions of nickel(II) and cobalt(II).

Acetic acid - sodium acetate buffer solution, pH 5·2.

Procedure—

Determination of nickel—Up to 6 p.p.m. of nickel, 15 ml of 0·1 per cent. *m/V* bipyridylglyoxal dithiosemicarbazone solution in ethanol, 20 ml of acetic acid - sodium acetate buffer

solution (pH 5·2) and up to 50 ml of water are poured into a separating funnel. The mixture is shaken briskly, left to stand for 30 minutes and then extracted four times with 5-ml volumes of chloroform. The chloroform extracts are collected in a 25-ml calibrated flask and diluted to the mark with chloroform. The absorbance at a wavelength of 410 nm is measured against a blank obtained by extraction of the reagents (containing no nickel) in the same way.

Determination of cobalt—A solution with a concentration of up to 8 p.p.m. of the cobalt is placed in a calibrated flask, 15 ml of 0·1 per cent. *m/V* bipyridylglyoxal dithiosemicarbazone solution in ethanol and 20 ml of pH 5·2 buffer solution are added and the mixture is diluted to 50 ml with water. The flask is shaken vigorously, allowed to stand for 1 hour and the absorbance is measured at 410 nm against a reagent blank.

Determination of nickel and cobalt in mixtures: Method A—A neutral solution of nickel(II) and cobalt(II) is poured into a separating funnel; the pH is maintained at 5·2 by use of the buffer solution. An excess of 0·1 per cent. *m/V* reagent solution is added. After 30 minutes the complexes are extracted into chloroform as indicated above. The aqueous and chloroform layers are separated and the absorbance of each is measured at 410 nm, as described. The amount of nickel is calculated from the absorbance of the organic layer, and cobalt from that of the aqueous layer.

Determination of nickel and cobalt in mixtures: Method B—Two identical aqueous sample solutions are prepared and in one of them the sum of nickel *plus* cobalt is determined at pH 5·2. With the other sample nickel is determined alone after extraction into chloroform. A calibration graph is plotted for the nickel ion in the aqueous layer and the amount of cobalt is obtained by difference.

Determination of nickel and cobalt in industrial catalysts—A 0·5-g amount of catalyst is weighed accurately and is placed in a flask that is suitable for refluxing to which 20 ml of concentrated nitric acid and 60 ml of concentrated hydrochloric acid are added. The mixture is refluxed for 1 hour and is then concentrated to about 25 ml. The solution is adjusted to pH 5 to 7 and diluted to 100 ml with distilled water in a calibrated flask. Aliquots are taken from this solution and the nickel and cobalt complexes are developed and the analysis is completed as described under *Method A*.

Results and Discussion

Reaction of bipyridylglyoxal dithiosemicarbazone with nickel and cobalt—

Bipyridylglyoxal dithiosemicarbazone forms yellow - green chelates with nickel(II) and cobalt(II) in a weakly acidic medium, the wavelengths of maximum absorption being 390 and 410 nm, respectively (Fig. 1). Both complexes are formed slowly and the solutions must be allowed to stand for a time for the development of a stable colour. Reducing agents do not

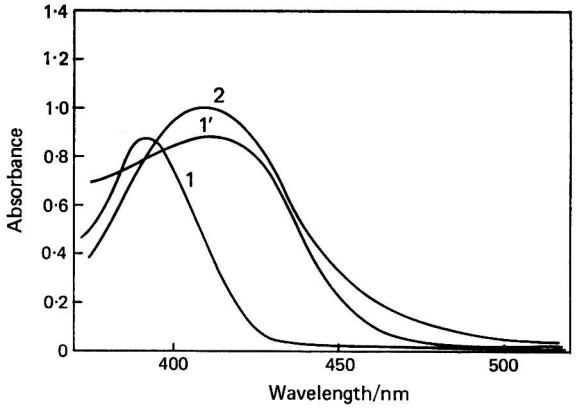


Fig. 1. Absorption spectra of solutions at pH 5·2 of complexes formed with bipyridylglyoxal dithiosemicarbazone: 1, 5 p.p.m. of nickel(II) in a homogeneous medium; 1′, 5 p.p.m. of nickel extracted into chloroform; and 2, 7 p.p.m. of cobalt(II) in a homogeneous medium

affect the cobalt complex spectrum, but oxidising agents, such as potassium persulphate and hydrogen peroxide, shift the maximum towards the ultraviolet, with a notable hyperchromic effect. The nickel complex is extracted into chloroform at pH 5·2 and the $\lambda_{max.}$ shifts to 410 nm. The cobalt complex is not extracted into this solvent at any pH value.

The different behaviour of nickel and cobalt complexes in regard to extraction into chloroform is probably caused by the charge on the cobalt complex. With iron(II), the bipyridylglyoxal dithiosemicarbazone - iron(II) complex is extracted into chloroform both in an acidic and in an ammoniacal medium, giving an emerald-green colour, but the extraction is easier in an ammoniacal medium. The extraction of other ions has not been tested, but their extraction is to be expected as some of them produce positive errors in the study of the interferences of the nickel(II) and iron(II) complexes. Iron(II) ions interfere in the determination of cobalt and nickel, as can be seen from the results of the study of the interferences of both ions.

The absorbance - pH graphs for the cobalt and nickel complexes are shown in Fig. 2. Despite its not being the optimum value, a pH of 5·2 has been chosen for the nickel complex because the sensitivity of the reaction is good, the acetic acid - sodium acetate buffer is more readily available, and some interferences are avoided at this pH value.

The absorbance of a chloroform solution of the nickel complex remains stable for at least 24 hours. The ethanol (from the reagent solution) extracted with chloroform stabilises the solutions.

The stoicheiometry of the complexes has been studied by the continuous variation method (Fig. 3). The reagent - metallic ion ratio found is 1:1 for the nickel complex and 2:1 for the cobalt complex.

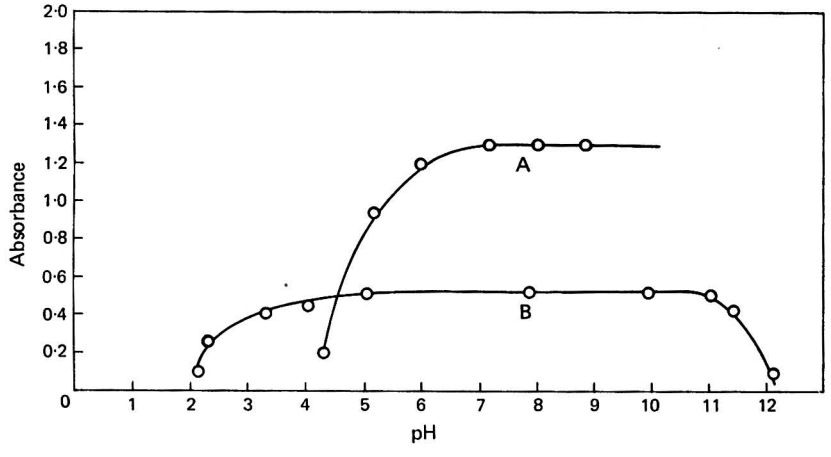


Fig. 2. Absorbance *versus* pH graphs of nickel and cobalt complexes of bipyridylglyoxal dithiosemicarbazone: A, nickel complex extracted into chloroform for 6 p.p.m. of nickel ($\lambda_{max.}$ 410 nm); and B, cobalt complex in homogeneous medium for 3·2 p.p.m. of cobalt ($\lambda_{max.}$ 410 nm). Both graphs have been obtained with use of various amounts of hydrochloric acid and sodium hydroxide

ANALYTICAL APPLICATIONS OF THE COBALT AND NICKEL COMPLEXES—

Nickel complex—The optimum conditions for the formation and extraction of the nickel complex have been indicated under Experimental. Beer's law is obeyed between 1 and 5 p.p.m. of nickel and the molar absorptivity at 410 nm is $1{\cdot}17 \times 10^4$ l mol^{-1} cm^{-1}. Ringbom's graph shows that 1·4 to 4·0 p.p.m. of nickel(II) is the minimum range of error. The relative error ($P = 0{\cdot}05$) of the method is $\pm$ 0·13 per cent.

The interferences on 4 p.p.m. of nickel have been investigated: 100 p.p.m. of cobalt(II), manganese(II), chromium(VI), silver, platinum(IV), tungsten(VI), lanthanum, calcium, barium and aluminium and 10 p.p.m. of mercury(II) ions gave errors below 5 per cent.; 2 p.p.m. of iron(II), copper(II), zinc, cadmium and osmium(IV) ions gave errors between 4 and 8 per cent.; 200 p.p.m. of fluoride, oxalate, citrate, phosphate and thiosulphate did not

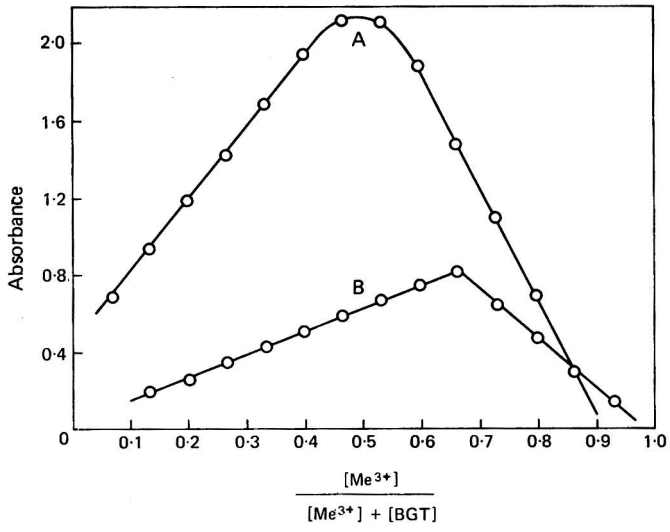


Fig. 3. Stoicheiometry of nickel and cobalt complexes of bipyridylglyoxal dithiosemicarbazone (continuous variations): A, nickel complex extracted into chloroform at pH 5·2 ($\lambda_{max.}$ 410 nm); and B, cobalt complex in homogeneous medium at pH 5·2 ($\lambda_{max.}$ 410 nm). The initial solutions of nickel and cobalt were at a concentration of 1×10^{-3} M

interfere or gave errors below 2 per cent.; and 5 p.p.m. of EDTA and cyanide gave an error of 10 per cent.

Cobalt complex—The conditions established in the recommended procedure have been determined empirically. Beer's law is obeyed at pH 5·2 for between 1 and 7 p.p.m. of cobalt and the molar absorptivity is $9{\cdot}05 \times 10^3$ l mol^{-1} cm^{-1}. The optimum concentration range, evaluated by Ringbom's method, is 1·5 to 7·5 p.p.m. of cobalt. The relative error ($P = 0{\cdot}05$) of the method is $\pm$ 0·55 per cent.

The interferences for 4 p.p.m. of cobalt were investigated: 100 p.p.m. of manganese(II), chromium(VI), platinum(IV), tungsten(VI), lanthanum, calcium, barium, aluminium, lithium, sodium, potassium, magnesium, rubidium and strontium ions did not interfere or gave errors lower than 2 per cent.; 10 p.p.m. of mercury(II) and cadmium ions, 2 p.p.m. of zinc and osmium(IV) ions and 0·5 p.p.m. of iron(II), copper(II) and nickel ions gave errors of over 15 per cent.; EDTA and cyanide ions interfered above 5 p.p.m.; and 200 p.p.m. of citrate gave errors of 5 per cent. At this concentration (200 p.p.m.) fluoride, oxalate, perchlorate, phosphate and thiosulphate do not interfere.

TABLE I

DETERMINATION OF NICKEL AND COBALT IN MIXTURES BY METHOD A

Determination of nickel			Determination of cobalt		
Nickel added/μg ml^{-1}	Ni:Co ratio	Nickel found/μg ml^{-1}	Cobalt added/μg ml^{-1}	Co:Ni ratio	Cobalt found/μg ml^{-1}
0·5	0·05	0·55	5·0	20·0	5·3
1·0	0·1	1·05	5·0	10·0	5·2
1·0	0·17	1·0	6·0	6·0	6·0
1·0	0·2	1·0	5·0	5·0	5·0
2·0	0·33	2·05	6·0	3·0	6·0
2·0	0·5	2·05	4·0	2·0	4·0
3·0	1·0	3·06	3·0	1·0	3·0
4·0	2·0	4·06	2·0	0·5	2·0
4·0	4·0	4·06	1·0	0·25	1·0
3·0	6·0	3·0	0·5	0·17	0·55
1·8	9·0	1·8	0·2	0·11	0·25

Analysis of nickel and cobalt mixtures—We have applied method A to a series of eleven samples in which the nickel to cobalt ratio varied from 0·05 to 9. The absorbance should not exceed 1. Table I shows the results obtained. It can be deduced from these results that nickel can be determined, within the concentration range given in Table I, in the presence of up to ten times its own concentration of cobalt with an error of less than 5 per cent. It is possible to determine cobalt in the presence of concentrations of nickel up to four times greater with errors below 4 per cent.

Table II shows the results obtained by use of method B when applied to thirteen samples in which the nickel to cobalt ratio varied from 0·14 to 7. In the presence of up to five times greater concentrations of one ion, the other can be determined with minimal error.

TABLE II

DETERMINATION OF NICKEL AND COBALT IN MIXTURES BY METHOD B

Nickel added/μg ml^{-1}	Nickel found/μg ml^{-1}	Cobalt added/μg ml^{-1}	Cobalt found/μg ml^{-1}
1·0	1·5	7·0	7·5
1·0	1·5	6·0	6·1
1·0	1·0	5·0	5·0
1·0	1·0	4·0	4·0
2·0	2·0	6·0	6·0
2·0	2·0	4·0	4·0
3·0	3·0	3·0	3·0
4·0	4·0	2·0	2·0
6·0	6·0	2·0	2·0
4·0	4·0	1·0	1·0
5·0	5·0	1·0	1·0
6·0	6·1	1·0	1·5
7·0	7·5	1·0	1·5

Analysis of industrial catalysts—The techniques described above have been applied to the determination of trace amounts of nickel and cobalt on aluminium oxide supports and in process catalysts such as the Unifining catalyst, Isomax-UOP-DHC-2, Filtrol 475–8 and Unifining Petresa. All of these supports have the shape of a ball of about 2 mm diameter, or a small cylinder of similar dimensions. For Unifining catalyst method A was used. The results are compared with those obtained by atomic-absorption spectrophotometry, and are shown in Table III. It should be noted that neither an excess of aluminium oxide nor the presence of molybdenum, which is present in these catalysts, interferes.

TABLE III

ANALYSIS OF INDUSTRIAL CATALYSTS

Sample*	Method†	Nickel, per cent.	Cobalt, per cent.
Unifining catalyst	AA	0·30	2·20
	BGT	0·33	2·20
Isomax-UOP-DHC-2	AA	0·82	—
	BGT	0·81	—
Filtrol 475–8..	AA	—	1·16
	BGT	—	1·16
Unifining Petresa	AA	2·31	—
	BGT	2·36	—

*Unifining catalyst: nickel 0·30 per cent., cobalt 2·20 per cent., molybdenum 3·40 per cent.
Isomax-UOP-DHC-2: nickel 0·82 per cent., molybdenum 4·60 per cent.
Filtrol 475-8: cobalt 1·20 per cent., molybdenum 2·45 per cent.
Unifining Petresa: nickel 2·35 per cent., molybdenum 4·50 per cent.
(According to standard of UOP.)
† AA = atomic absorption; BGT = bipyridylglyoxal dithiosemicarbazone.

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Received *July 11th*, 1973
Accepted *December 28th*, 1973

Analyst, June, 1974, Vol. 99, *pp.* 360–366

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

Part VI.* The Determination of Thiols

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Alkyl and aryl thiols have been determined in the presence of carboxylic acids and phenols by means of acid - base catalytic thermometric titrimetry. Two titrations are carried out, with acrylonitrile and acetone as the end-point indicators. With the former indicator, thiol groups are not determined, so that the difference between the titration values obtained by using the two methods of end-point indication is a measure of the thiol content.

The thiol content of 2-mercaptothiazoline, 4,6-dihydroxypyrimidine-2-thiol (2-thiobarbituric acid), purine-6-thiol and 2-mercaptobenzimidazole can be determined by the same procedure. In the titration of 2-thiohydantoin, 4-hydroxypyrimidine-2-thiol (2-thiouracil), 2-mercaptobenzoxazole and 2-mercaptobenzothiazole, however, both end-point methods give the same titration value. These apparently anomalous results can be explained if it is accepted that the last four heterocyclic thiols exist in the thione tautomeric form in dimethylformamide solution. Some thioamides also titrate as acids, and differences between titration values obtained by using the two methods of end-point indication can again be attributed to thione - thiol tautomerism.

Thiols can be determined conveniently in amounts down to 0·01 mequiv, *i.e.*, about 2 mg of dodecane-1-thiol, with 0·1 M titrants. In instances when the acrylonitrile method can be used for the direct determination of the thiol function, 0·001 M titrant can be used and the lower level of determination is then about 0·0001 mequiv.

The determination of the thiol function is important in connection with the refining of petroleum because thiols are an undesirable impurity in distillate fractions. In other industries, certain thiols find use as additives. For example, dodecane-1-thiol is used to control the degree of polymerisation in the manufacture of thermoplastics, while 2-mercaptobenzothiazole is added to rubber formulations as an accelerator for the vulcanisation stage. The performance of such additives is dependent on their thiol content, and determination of the latter can be used as a method of quality control.

Thiols differ from hydroxy compounds in their ability to form insoluble copper, silver and mercury derivatives and in the ease with which they undergo oxidation. These reactions form the basis of the preferred chemical methods for the determination of the thiol function in organic compounds.[1,2] Thiols are more acidic than the corresponding hydroxy compounds, thus even ethanethiol, with a pK_a of 10·5 at 20 °C, can be titrated as a weak acid.[2] Acid - base titration is not, however, generally recommended for the selective determination of thiols because other acidic compounds in the sample would interfere.

An attempt has been made to determine thiols iodimetrically in non-aqueous solution by use of catalytic thermometric titration with ethyl vinyl ether as an end-point indicator, but the titration values obtained were considerably lower than those required by a 1:1 stoicheiometry.[3] Apparently, a high proportion of the thiol was converted into an unreactive, *i.e.*, unoxidisable, sulphide by addition to the indicator monomer during the course of the titration:

$$-SH + C_2H_5OCH{=}CH_2 \rightarrow C_2H_5OCH_2CH_2S-$$

* For Part V of this series, see *Analyst*, 1974, **99**, 82.

In the present paper an analytical procedure is reported in which the acid content of a sample is determined by two catalytic thermometric methods, the acrylonitrile method described in Part II[4] and the acetone-indicator method of Vaughan and Swithenbank.[5] With acrylonitrile as the end-point indicator thiol groups that undergo rapid addition to acrylonitrile are not determined, and the difference between the titration values obtained by using the two end-point indicators is taken as being a measure of the content of these thiol groups. This procedure offers an alternative to the two preferred methods, noted above, for the selective determination of the thiol group, and it has been evaluated for a range of alkyl, aryl and heterocyclic thiols and for some thioamides.

Experimental

Reagents—

Acetone, propan-2-ol and methanol were analytical-reagent grade materials, and acrylonitrile, toluene and dimethylformamide were laboratory-reagent grade materials. All were dried over molecular sieve 4A before use. Other solvents and reagents, including the thiols, were laboratory-reagent grade materials and were used as received.

Potassium hydroxide, 1·0 and 0·1 M solutions in propan-2-ol—Standardise these solutions against benzoic acid (analytical-reagent grade) in acetone by the thermometric method using acetone as the end-point indicator.

Tetra-n-butylammonium hydroxide, 0·1 M solution in toluene - methanol—Laboratory-reagent grade material was used as received. Prepare 0·01 and 0·001 M solutions by adding appropriate volumes of toluene - propan-2-ol mixture (3 + 1) to the 0·1 M reagent. Standardise the solutions against benzoic acid (analytical-reagent grade) dissolved at 0·1 or 0·01 N concentration in dimethylformamide by the thermometric method with acrylonitrile as the end-point indicator.

Apparatus—

Use a motor-driven syringe to supply the titrant, a thermistor to measure the temperature, and a 10-ml titration flask with a magnetic stirrer, as described in Part III.[6]

Procedure

A. Acetone-indicator method—

Use the procedure described by Vaughan and Swithenbank[5] in the following manner. Add potassium hydroxide titrant solution at a rate of 0·1 ml min^{-1} to a mixture of 1 ml of sample solution and 3 ml of acetone in the titration flask; use 1·0 mequiv of sample with the 1·0 M titrant and 0·1 mequiv of sample with the 0·1 M titrant. Record the temperature and titrant volume on a millivolt chart recorder (50 and 20-mV scales with the 1·0 and 0·1 M titrants, respectively) at a chart speed of 600 mm h^{-1}.

B. Acrylonitrile-indicator method—

Use the procedure described in Part IV[7] for the titration of acidic functions. Dissolve the sample in 1 ml of dimethylformamide and use 0·1 mequiv of sample with the 0·1 M tetra-n-butylammonium hydroxide titrant or correspondingly smaller amounts with the 0·01 and 0·001 M titrants; 1·0 or 0·1 M potassium hydroxide solution can also be used as a titrant.

The end-point of the titration, when either indicator method is used, is located at the point where the tangent to the main heat rise leaves the curve at its lower temperature end.[8]

Results and discussion

Table I lists the compounds titrated and the reaction stoicheiometries obtained by using the acetone and acrylonitrile-indicator methods. Some thioamides that can be titrated as acids are included in the table.

Titration curves obtained in the determination of some of the thiols and thioamides are shown in Figs. 1 and 2, respectively.

It can be seen from Table I that the monofunctional alkyl and aryl thiols are not determined by the acrylonitrile-indicator method. Thus, the addition of acrylonitrile to these compounds, *i.e.*, cyanoethylation, must have proceeded to completion before the acid - base

reaction could occur. Cyanoethylation is catalysed by the alkaline titrant:

$$RSH + CH_2{=}CHCN \xrightarrow{OH^-} RSCH_2CHCN$$

The carboxylic and phenolic groups of 2-mercaptobenzoic acid and salicylideneaminobenzene-2-thiol are determined, and the difference between the titration value for each compound and the corresponding titration value obtained by using the acetone-indicator method, which determines the thiol function also, is a measure of the thiol content.

TABLE I

THIOLS AND THIOAMIDES TITRATED WITH 1·0 M POTASSIUM HYDROXIDE AND 0·1M TETRA-n-BUTYLAMMONIUM HYDROXIDE SOLUTIONS WITH ACETONE AND ACRYLONITRILE, RESPECTIVELY, AS END-POINT INDICATORS

Conditions: titrate 1 mequiv of thiol in 3 ml of acetone with 1·0 M potassium hydroxide solution by using the acetone-indicator method, and 0·1 mequiv of thiol in a mixture of 1 ml of dimethylformamide and 2 ml of acrylonitrile with 0·1 M tetra-n-butylammonium hydroxide solution by using the acrylonitrile-indicator method

Aliphatic thiols—
Heptane-1-thiol (1:0·9:0); dodecane-1-thiol (1:0·9:0); 2,3-dimercaptopropan-1-ol (2:1·7:0); and mercaptosuccinic acid (3:2·5:1·9)

Aromatic thiols—
Toluene-1'-thiol (1:0·8:0); toluene-4-thiol (1:0·8:0); 4-aminobenzenethiol (1:0·65:0); 2-mercaptobenzoic acid (2:2:1); salicylideneaminobenzene-2-thiol (2:1·9:1); and pyridine-2-thiol (1:1:0·18)

Heterocylic thiols—
2-Mercaptothiazoline (1:1:0); 2-thiohydantoin (1:1:1); 4-hydroxypyrimidine-2-thiol (2:1:1); 4,6-dihydroxypyrimidine-2-thiol (3:2:1); purine-6-thiol (1:1·7:0·8); 2-mercaptobenzimidazole (1:1:0·3); 2-mercaptobenzoxazole (1:1:1); and 2-mercaptobenzothiazole (1:1:1)

Thioamides—
Thioacetamide (1:1:0·9*); thiourea (1(2):0·1:1*); thiocarbanilide (1(2):1:0·36 or 0·53*); dithiooxamide (rubeanic acid) (2:2:1); thiosemicarbazide (1(2):1·1:0·9); and diphenylthiocarbazone (dithizone) (1(2):1:0·8 or 1*)

Figures in parentheses following the name of the compound denote the theoretical number of acidic functional groups in the molecule, the number of groups titrated by using the acetone-indicator method and the number of groups titrated by using the acrylonitrile-indicator method, respectively.

* Values obtained in the titration of 1 mequiv of sample by using the acrylonitrile-indicator method and 1·0 M potassium hydroxide titrant.

The content of thiols in mixtures with carboxylic acids and phenols can also be determined by using the two indicators. In Fig. 3 calibration graphs are shown for mixtures of dodecane-1-thiol with benzoic acid and 3,5-xylenol with 2-mercaptothiazoline. The irregular shape of the calibration graph for the mixture containing dodecane-1-thiol is due to the low reaction stoicheiometry with this compound.

It can be seen from Table I that the titration reactions with several of the thiols are sub-stoicheiometric, and it is necessary to use calibration graphs, or to allow for the sub-stoicheiometry in some other way, when determining these thiols by the suggested procedure.

The small but finite titration value obtained when pyridine-2-thiol is determined by using the acrylonitrile-indicator method indicates that the rate of cyanoethylation is influenced by the heterocyclic ring. The titration values obtained with the other heterocyclic thiols examined have been found to depend on the position of the thiol group in the molecule, or the presence of reactive functional groups in addition to the thiol group, or both. Thus, while 2-mercaptothiazoline behaves on titration in the same way as do simple alkyl and aryl thiols, the corresponding benzo-derivative, 2-mercaptobenzothiazole does not. The thiol group in the latter compound can, apparently, be determined by either of the two catalytic end-point methods. This anomaly can be explained if it is assumed that, in solution in dimethylformamide, 2-mercaptobenzothiazole exists entirely in the thione tautomeric form. In this form it is the acidic imido group and not the thiol group that is titrated. The reaction

stoicheiometry of 2-mercaptobenzoxazole can be similarly explained. This proposed reaction path presupposes that the imido group undergoes cyanoethylation more slowly than it undergoes neutralisation by the titrant.

In contrast with its sulphur and oxygen analogues, 2-mercaptobenzimidazole was found to give a titration value corresponding to a sub-stoicheiometric reaction when it was determined by using the acrylonitrile-indicator method. This would suggest that the compound is only partly in the thione form when in solution in dimethylformamide.

2-Thiohydantoin and 2-thiouracil (4-hydroxypyrimidine-2-thiol) were also found to be titrated as monobasic acids irrespective of the end-point indicator used in the thermometric titration. With these two compounds, however, there is the possibility that the hydroxyl group in the enolic form of the molecules rather than the imido group, is being titrated.

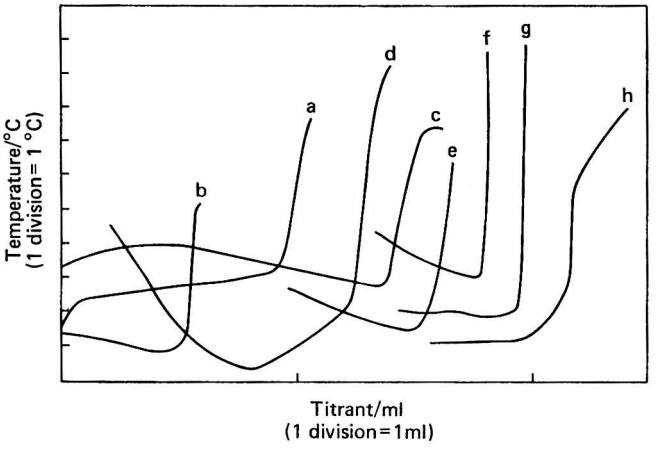

Fig. 1. Thermometric titration curves obtained in the determination of thiols by the acetone and acrylonitrile-indicator methods

	a*	b	c*	d*	e	f	g	h
Compound/mg ..	A, 205·5	B, 1·67	C, 105·9	D, 212·5	E, 7·8	F, 3·7	G, 6·4	H, 7·1
Solvent/ml ..	K, 2	D, 1	K, 2	K, 1	D, 1	D, 1	D, 1	D, 1
Titrant/M ..	P, 1·0	B, 0·01	P, 1·0	P, 1·0	B, 0·1	B, 0·1	B, 0·1	B, 0·1
Indicator method	K	P	K	K	P	P	P	P

Compounds—A, dodecane-1-thiol; B, 2-mercaptobenzothiazole; C, 2,3-dimercaptopropan-1-ol; D, salicylideneaminobenzene-2-thiol; E, 2-thiobarbituric acid; F, mercaptosuccinic acid; G, 2-thiouracil; and H, 2-thiohydantoin

Solvents—K, acetone; and D, dimethylformamide

Titrants—P, potassium hydroxide reagent; and B, tetra-n-butylammonium hydroxide reagent

Indicator methods—K, acetone method using 3 ml of acetone; and P, acrylonitrile method using 2 ml of acrylonitrile

* 1 division of temperature scale = 0·5 °C

Thiobarbituric acid (4,6-dihydroxypyrimidine-2-thiol), when determined by the thermometric titration, functions either as a dibasic or monobasic acid, depending on whether the acetone or acrylonitrile method of end-point indication is used. Barbituric acid (2,4,6-trihydroxypyrimidine), in contrast, behaves as a dibasic acid whichever method of end-point indication is used, thereby supporting the hypothesis that thiol groups cannot be determined in the presence of acrylonitrile.

Purine-6-thiol is similar to thiobarbituric acid with respect to the acidity it displays on catalytic thermometric titration, but the corresponding stoicheiometries, 1·7 and 0·8, are less well defined.

Although the thioamides are formally classed as thiones, they can undergo tautomeric change to thienols. The stoicheiometries of the neutralisation reactions in which dithio-

oxamide and thiocarbanilide are determined by using the two methods of end-point indication suggest that, in solution in dimethylformamide, the former compound has the structure $S:C(NH_2).C(SH):NH$, while the latter is partly in the thienolic form, $C_6H_5NH.C(SH):NH.C_6H_5$.

Thioacetamide, thiosemicarbazide and diphenylthiocarbazone (dithizone) would appear to retain the thione structure in solution in dimethylformamide, as a stoicheiometry of 1:1, or nearly 1:1, is obtained when either method of end-point indication is used.

Thiourea behaves in an unusual way in the thermometric titration in that, while the simple 1:1 stoicheiometry is obtained by using the acrylonitrile indicator, the neutralisation proceeds to only a small extent when acetone is used to mark the end-point.

Both thiourea and thioacetamide show an immediate temperature rise when they are titrated in the presence of the acrylonitrile end-point indicator, but the neutralisation then proceeds and an S-shaped titration curve results (Fig. 2). The curves are similar in shape to those obtained in the titration of slightly soluble catecholamines[7] but, as thiourea and thioacetamide are readily soluble, the shape must be due to some other factor. A possible answer is that the initial addition of titrant causes a rearrangement of either the thioamide or an unstable addition compound of the thioamide and acrylonitrile. It should be noted (Table I) that, for these two amides, 1·0 M potassium hydroxide solution was used as the titrant instead of 0·1 M tetra-n-butylammonium hydroxide solution. With the latter titrant the titration curves were rounded and the end-point was difficult to assess.

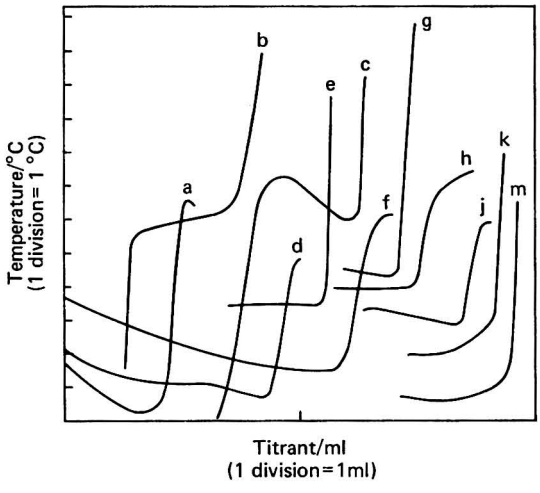

Fig. 2. Thermometric titration curves obtained in the determination of thioamides by the acetone and acrylonitrile-indicator methods

	a*	b	c	d*	e	f*	g	h	j	k*	m
Compound/mg ..	A,31·0	A,48·4	B,43·5	C,54·1	C,5·96	D,278·0	D,17·2	E,3·2	E,36·8	F,83·7	F,117·4
Solvent/ml ..	D, 1	D, 1	D, 1	K, 1	D, 1	K, 1	D, 1	D, 1	K, 2	D, 1	D, 2
Titrant/M ..	P, 1·0	P, 1·0	P, 1·0	P, 1·0	B, 0·1	P, 1·0	B, 0·1	P, 0·1	P, 1·0	P, 1·0	P, 1·0
Indicator method	K	P	P	K	P	K	P	P	K	K	P

Compounds—A, thioacetamide; B, thiourea; C, dithiooxamide; D, thiocarbanilide; E, thiosemicarbazide; and F, diphenylthiocarbazone

Solvents—K, acetone; and D, dimethylformamide

Titrants—P, potassium hydroxide reagent; and B, tetra-n-butylammonium hydroxide reagent

Indicator methods—K, acetone method using 3 ml of acetone; and P, acrylonitrile method using 2 ml of acrylonitrile

* 1 division of temperature scale = 0·5 °C

When 1·0 M potassium hydroxide titrant was used in conjunction with the acrylonitrile method for the determination of thiocarbanilide and diphenylthiocarbazone, the stoicheiometries of the neutralisation reactions differed from those obtained when the tetra-n-butyl-

ammonium hydroxide reagent was used, and the possibility of steric hindrance influencing the stoicheiometry must be considered.

The precision of the method for the determination of thiols in the presence of carboxylic acids and phenols has been determined for mixtures of dodecane-1-thiol and benzoic acid, and 2-mercaptothiazoline and 3,5-xylenol, by carrying out eight titrations on each mixture dissolved in dimethylformamide against 1·0 and 0·1 M potassium hydroxide titrants, respectively. The acetone and acrylonitrile-indicator methods were used alternately as the titrations were carried out.

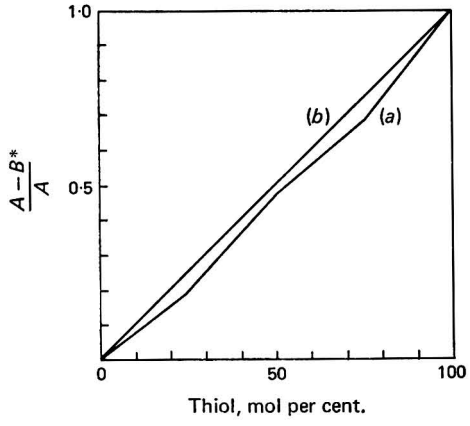

Fig. 3. Calibration graphs for the thermometric titration of mixtures of dodecane-1-thiol with benzoic acid, and 2-mercaptothiazoline with 3,5-xylenol. (*a*), Dodecane-1-thiol *plus* benzoic acid, 1 mequiv dissolved in 2 ml of dimethylformamide; and (*b*), 2-mercaptothiazoline *plus* 3,5-xylenol, 0·1 mequiv dissolved in 1 ml of dimethylformamide. 1·0 and 0·1 M potassium hydroxide titrants, respectively, were used in the determinations of mixtures (*a*) and (*b*).

**A* and *B* are the titration values obtained by using the acetone-indicator method with 3 ml of acetone and the acrylonitrile-indicator method with 2 ml of acrylonitrile, respectively

Precisions were calculated in two ways: (*a*), from the differences between pairs of titration values taken in sequence; and (*b*), from the precisions of the groups of four titration values obtained by each method of end-point indication. In (*b*) the required coefficient of variation has been calculated as the square root of the sum of the squares of the coefficients of variation of the two groups of four titrations. Details of the experimental results and the calculated values are shown in Table II. It can be seen that the direct determination of the coefficient of variation from the differences between pairs of titration values leads to lower values than by calculation using method (*b*) and there is, therefore, apparently some advantage in carrying out the titrations in the sequence proposed.

The method is suitable for the determination of thiols in the presence of carboxylic acids and phenols at precisions of about 1 per cent., and in amounts down to 0·01 mequiv when 0·1 M titrants are used, provided that the thiol function is titrated when the acetone-indicator method is used, but is not titrated in the acrylonitrile-indicator method. This procedure requires that two titrations be carried out with each sample but, of course, gives the content of acidic compounds other than thiols as well as the thiol content.

Values for the precisions of the titration values obtained in the direct determination of the thiol function in some other compounds are shown in Table III, and it can be seen that these values are of the same order, *i.e.*, 0·32 to 2·2 per cent., as those obtained previously in acid - base titrations in which acrylonitrile was used as the end-point indicator.[4] When this

Table II

Results for precision from the thermometric titration of mixtures of dodecane-1-thiol with benzoic acid, and 2-mercaptothiazoline with 3,5-xylenol, against 1·0 and 0·1 M solutions of potassium hydroxide

Titration results—

Method*		A	B	A	B	A	B	A	B
Compounds/mg†									
(*i*) Dodecane-1-thiol, 81·2 *plus* benzoic acid, 48·2	Titre/ml	0·683	0·390	0·681	0·391	0·678	0·388	0·678	0·391
	A–B/ml	0·293		0·290		0·290		0·287	
(*ii*) 2-Mercaptothiazoline, 5·9 *plus* 3,5-xylenol, 6·1	Titre/ml	1·004	0·492	1·012	0·494	1·016	0·500	1·016	0·496
	A–B ml	0·512		0·518		0·516		0·520	

Coefficients of variation of the differences in titration values obtained by using methods A and B—

	Directly from the differences in sequential titration values, per cent. [method (*a*)]	Indirectly from the individual coefficients of variation of methods A and B, per cent. [method (*b*)]
Mixture (*i*) ..	0·99	1·19
Mixture (*ii*) ..	0·67	0·90

* A, acetone-indicator method; B, acrylonitrile-indicator method.
† Dissolved in 1 ml of dimethylformamide.

end-point indicator is suitable for the direct determination, it is possible to use 0·001M titrants and to determine thiols in amounts down to about 0·0001 mequiv, although this is possible only if other acidic functions are absent.

Table III

Results for precision from the thermometric titration of thiols and thioamides with 1·0 and 0·1 M potassium hydroxide and 0·1 M tetra-n-butylammonium hydroxide reagents

Thiol or thioamide	Amount/ mg	Titration method*	Titrant†/ M	*n*‡	Mean titre/ml	Standard deviation	Coefficient of variation, per cent.
2-Mercaptobenzothiazole	14·8	P	B, 0·1	3	0·87	0·004	0·46
2-Mercaptobenzothiazole	0·17	P	B, 0·001	4	1·31	0·029	2·20
2-Thiohydantoin	7·12	P	B, 0·1	4	0·58	0·003	0·52
Mercaptosuccinic acid ..	7·40	P	B, 0·1	3	0·94	0·003	0·32
2-Mercaptobenzoic acid ..	67·5	A	K, 1·0	3	0·84	0·007	0·83
4-Aminobenzenethiol ..	163·5	A	K, 1·0	3	0·88	0·005	0·57
Dithiooxamide	6·0	P	B, 0·1	3	0·97	0·005	0·50

* P, acrylonitrile indicator; A, acetone indicator.
† B, tetra-n-butylammonium hydroxide reagent; K, potassium hydroxide reagent.
‡ Number of determinations.

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NOTE—References 4, 6 and 7 are to Parts II, III and IV of this series respectively.

Received *December 4th*, 1973
Accepted *January 11th*, 1974

Analyst, June, 1974, Vol. 99, *pp.* 367–375

Determination of Ammonia Levels in Water and Wastewater With an Ammonia Probe

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The application of an ammonia probe has been investigated for discrete laboratory measurement of ammonia levels in a variety of waters. The probe displays a Nernstian response for the range 0·2 to 40 mg l^{-1} of ammoniacal nitrogen in a stirred 0·1 M sodium hydroxide solution containing 0·01 M ethylenediaminetetraacetic acid. Recoveries of added ammonia from a wide range of water samples are satisfactory. Both these recoveries of added ammonia and repeated calibrations of the probe suggest a precision of 4 per cent. for ammoniacal nitrogen concentrations greater than 0·4 mg l^{-1} and 0·015 mg l^{-1} for concentrations less than 0·4 mg l^{-1}; the statistical limit of detection is 0·03 mg l^{-1}. Good agreement is obtained with existing methods based on distillation and spectrophotometric measurement for a further range of samples, but the limit of detection and the precision at low levels suggest that accurate determination in potable waters would be difficult.

The probe can also be used to determine albuminoid nitrogen by taking the difference between the ammoniacal nitrogen and the total free *plus* albuminoid nitrogen obtained by distillation. Values obtained in this way agree with those obtained by existing methods subject to the precision of the probe being acceptable.

Free and saline ammonia, expressed as ammoniacal nitrogen, is normally determined in the course of examination of water and wastewater; less frequently, albuminoid nitrogen is also determined. Ammoniacal nitrogen may vary from a negligible level in potable water (less than 0·01 mg l^{-1}) to values exceeding 40 mg l^{-1} in settled sewage. The recommended methods for its determination in potable and wastewaters involve distillation of the ammonia from an alkaline medium, magnesium oxide[1] or sodium carbonate,[2] or from a phosphate buffer,[3] followed by spectrophotometric or titrimetric measurement of the distilled ammonia. The albuminoid nitrogen is determined subsequently on the same sample by distillation from alkaline permanganate, with the same means of measurement.

A commercial probe introduced recently measures ammonia directly and has been used to determine free ammonia levels in boiler feed-waters.[4] An investigation into the possible laboratory application of this probe to the determination of ammoniacal and albuminoid nitrogen in swimming-pool and raw potable waters and wastewaters is described in this paper.

Experimental

The ammonia probe used in this work was an Electronic Instruments Ltd. (EIL) Laboratory Model 8002-2, consisting of a glass pH electrode surrounded by a filling solution of ammonium chloride, in contact with a gas-permeable hydrophobic membrane. Free ammonia diffuses through this membrane until the partial pressures in the sample on the one side and the thin film of standard ammonium chloride filling solution on the other side are equal. When this steady state is reached, the ammonia - ammonium ion equilibrium in the thin film gives a value for the hydrogen-ion concentration, which is monitored by changes in potential of the glass pH electrode, referred to a silver - silver chloride electrode also dipping into the filling solution.

Alkaline pH adjustment—

Ammonium ions are converted into free ammonia exclusively at pH values exceeding 12. A dilution of 10 parts of a standard solution (see Reagents) with 1 part of 1 M sodium hydroxide solution or 1 part of 0·1 M sodium hydroxide solution produced an average difference in readings of 1·6 mV (Table I). This difference could not be explained by the presence of any free

ammonia in the sodium hydroxide solution as potentials obtained with standards adjusted with boiled and unboiled 1 M alkali solution were the same. Hence, the difference may be due to the buffering effect of de-ionised water giving a final pH of less than 12. Ethylenediaminetetraacetic acid was added to the pH adjuster to prevent precipitation of hydroxides (mainly magnesium), thereby avoiding deposition on glass surfaces or on the electrode membrane; this addition produced no significant difference in millivolt readings (Table I).

TABLE I

EFFECT OF DIFFERENT ALKALI CONDITIONS ON PROBE RESPONSE

Results are meter readings in millivolts

Alkali conditions*	Ammoniacal nitrogen/mg l^{-1} 2	1	0·4	0·2	0·1
0·1 M Sodium hydroxide	+23·6	+40·0	+63·2	+79·2	+92·2
1·0 M Sodium hydroxide	+21·8	+39·2	+61·8	+77·2	+90·2
1·0 M Sodium hydroxide (boiled)	+22·0	+39·2	+61·8	+77·2	+90·2
1·0 M Sodium hydroxide *plus* 0·1 M EDTA ..	+22·0	+39·0	+62·4	+77·2	+90·2
1·0 M Sodium hydroxide *plus* 0·1 M EDTA (boiled)	+22·0	+39·4	+62·0	+77·2	+90·0

* 5 ml of alkaline solution used with 50 ml of standard solution.

TABLE II

EFFECT OF DIFFERENT TOTAL IONIC CONCENTRATIONS ON DETERMINATION AT DIFFERENT ALKALI STRENGTHS

Results are meter readings in millivolts

Concentration of added sodium chloride/M	Ammoniacal nitrogen/mg l^{-1} 40	10	4	1	0·4	0·1
Alkali addition of 1 M *NaOH* + 0·1 M *EDTA*—						
0	−57·8	−21·8	+2·0	+36·6	+59·8	+87·8
0·01	−57·8	−21·8	+2·0	+36·8	+60·0	+87·2
0·02	−58·0	−21·8	+2·0	+36·6	+59·4	+87·8
0·05	−57·8	−21·8	+2·0	+36·6	+60·0	+87·8
Alkali addition of 0·1 M *NaOH* + 0·01 M *EDTA*—						
0	−55·8	−20·4	+3·4	+38·0	+61·8	+91·0
0·01	−55·2	−20·2	+3·4	+38·0	+61·8	+90·6
0·02	−54·8	−20·2	+3·6	+38·6	+61·2	+90·4
0·05	−53·8	−19·8	+4·0	+38·8	+61·2	+91·4

Because the membrane is hydrophobic, ions cannot interfere directly; however, a high ionic concentration can affect the partial pressure of ammonia. It is therefore desirable to maintain the total ionic strengths within limits. Potable and surface waters seldom have total dissolved solids exceeding 1000 mg l^{-1} (about 0·02 M, calculated as sodium chloride). The response to variation in the total ionic strength at the two alkali concentrations was tested by addition of sodium chloride to standard solutions containing the higher strength alkali pH adjuster, at nominal total ionic strengths of 0·13, 0·14, 0·15 and 0·18, and similarly for the lower strength alkali pH adjuster, to give nominal total ionic strengths of 0·01, 0·02, 0·03 and 0·06. The results shown in Table II indicate that the probe is more susceptible to changes in the total ionic strength at the lower alkali concentrations, particularly at the higher ammonia concentrations. For this reason, and because of the possibility of the buffering effect of natural waters giving final pH values less than 12, it is recommended that the higher strength alkali pH adjuster should be used.

RESPONSE TIME—

For the experimental purposes described herein, equilibrium response was taken to satisfy the criterion of a shift of less than 0·2 mV min^{-1} at constant temperature. The time taken to achieve equilibrium response for stepwise change in ammoniacal nitrogen concentration, as illustrated in Tables I and II, was 4 minutes above 1 mg l^{-1} and 8 minutes in the range 0·1 to 1·0 mg l^{-1}. Below 0·1 mg l^{-1} readings should be taken after 10 minutes; longer equilibrium times would result in loss of ammonia from the alkaline solutions.

It has been reported that, providing measurement is made after immersion in distilled water, the probe attains an equilibrium potential within 2 to 5 minutes.[4] For samples from different origins varying widely in ammoniacal nitrogen content, the time required for the necessary equilibration in distilled water between each sample would detract from the value of using the probe. The millivolt readings obtained for changes in the range 0·1 to 100 mg l^{-1} of ammoniacal nitrogen for 1000, 100 and 10-fold increases and decreases in concentration are shown in Table III; readings were measured after the times specified for duplicate standard solutions at each particular level. These results indicate that hysteresis occurs when measurements are made on successive solutions decreasing more than 10-fold in concentration because of the diffusion of ammonia from the electrode internal filling solution, after immersion in a high concentration solution, into the low concentration sample solution. It is therefore advisable, when consecutive readings from a higher to a lower concentration differ by more than a decade, *i.e.*, by an absolute potential greater than 60 mV, that a repeat measurement be made.

TABLE III

STABILITY OF RESPONSE TO 1000-, 100- AND 10-FOLD INCREASE OR DECREASE IN AMMONIACAL NITROGEN LEVELS

Results are meter readings in millivolts

	Change in concentration of ammoniacal nitrogen/mg l^{-1}				
	100 to 0·1	10 to 0·1	1·0 to 0·1	100 to 10	10 to 1
Descending order	+66·0, +77·2	+76·0, +80·6	+80·8, +80·6	−29·4, −29·2	+28·0, +28·2
Ascending order	−88·0, −88·6	−29·8, −29·4	+28·8, +28·8		

CALIBRATION—

A recent method for the determination of ammonia with this probe has involved the use of a calibration graph prepared by plotting the differences in the potential of standards from that of a reference standard concentration against the logarithm of the concentration measured.[4] Samples are similarly measured against the reference standard concentration and ammonia levels read from the calibration graph. With frequent calibration of the reference concentration any drift in potential is avoided. The application of this probe to the measurement of ammonia in the wider range of samples envisaged would require, however, consistent differences from a reference standard in the range 0·01 to 40 mg l^{-1} of ammoniacal nitrogen.

The level of ammonia in de-ionised water is variable and may be as high as 0·01 mg l^{-1}. Treatment of de-ionised water with a strong cationic exchanger produced a water containing a consistent level of 0·002 mg l^{-1} of ammoniacal nitrogen, determined by distillation and spectrophotometric measurement. This treated water was used to prepare all standards at concentrations less than 1·0 mg l^{-1}.

The potential differences of standards in the range 0·01 to 40 mg l^{-1} of ammoniacal nitrogen with reference to a 1 mg l^{-1} standard showed no change during a probe life of 30 days, although the absolute potentials drifted in a positive direction. The criterion of a probe life of 30 days was accepted for all values obtained, the probe membrane being readily replaceable. The calibration graph was essentially linear in the range 0·2 to 40 mg l^{-1} of ammoniacal nitrogen, with a Nernstian response of 58·5 mV per decade difference, which was also obeyed up to a concentration of 100 mg l^{-1}. Average millivolt differences from the standard concentration at each level, together with the decade differences and the standard deviations from the means expressed also in terms of ammoniacal nitrogen, are shown in Table IV. This table includes the readings from 16 calibration runs taken during a membrane life of 30 days and readings from 40 calibration runs taken during a period of 6 months, during which the probe membrane was changed five times.

The average calibration readings for the 1- and 6-month periods are in agreement, indicating satisfactory stability for the probe. Thus, a calibration graph, once prepared, could be used repeatedly with only an occasional check when the probe membrane is changed or to ascertain the condition of the probe. It is noticeable that at ammoniacal nitrogen concentrations of less than 1·0 mg l^{-1}, averages differ depending on whether the calibration is conducted in an ascending or descending order of concentration. This is because of the hysteresis

TABLE IV

STATISTICAL SURVEY OF CALIBRATION OF AMMONIA PROBE
Results are meter readings in millivolts

	Number of readings	Ammoniacal nitrogen/mg l^{-1} 40	20	10	4	2	1	0·4	0·2	0·102	0·042	0·022	0·012
Average of values during 6 months	40	−94·0	−76·3	−58·4	−35·1	−17·3	0	+23·5	+38·9	+53·4	+66·0	+75·1	+78·8
Average of values during 1 month	16	−94·2	−76·4	−58·6	−35·0	−17·5	0	+23·9	+39·3	+53·8	+67·3	+75·8	+78·5
Average of ascending values during 1 month	8	−94·1	−76·4	−58·6	−35·2	−17·6	0	+24·3	+39·8	+54·7	+70·5	+78·3	+81·4
Average of descending values during 1 month	8	−94·3	−76·4	−58·5	−34·8	−17·3	0	+23·5	+38·7	+52·8	+64·3	+73·4	+75·6
Decade difference during 6 months	40	—	—	—	58·9	59·0	58·4	58·6	56·2	53·4	42·5	36·2	25·4
Decade difference during 1 month	16	—	—	—	59·2	58·9	58·6	58·9	56·8	53·8	43·4	36·5	24·7
Standard deviation from mean during 6 months	40	±0·90	±0·75	±0·80	±0·75	±0·50	—	±0·75	±1·5	±1·9	±4·2	±3·3	±4·9
Standard deviation from mean during 1 month	16	±0·90	±0·75	±0·65	±0·65	±0·30	—	±0·70	±1·2	±1·9	±4·0	±4·2	± 5·0
Standard deviation from mean during 6 months, as ammoniacal nitrogen/mg l^{-1}	40	1·6	0·6	0·4	0·15	0·05	—	0·01	0·015	0·010	0·015	0·010	0·012

effect mentioned previously and is very marked at concentrations less than 0·1 mg l^{-1}. It appears that measurement at these low levels should be conducted after stabilisation in ammonia-free water, but the precision of ascending order of standards around the respective means at these levels is similar to the over-all precisions indicated in Table IV. To break the standard procedure is therefore unlikely to confer advantages for these low levels.

The precision at each level of standards suggests that concentrations of ammoniacal nitrogen greater than 0·4 mg l^{-1} should be measurable to an accuracy within 4 per cent. and concentrations less than 0·4 mg l^{-1} to within 0·015 mg l^{-1}. A statistical limit of sensitivity of 0·03 mg l^{-1} was calculated from these readings.

The temperature during these measurements varied between 22 and 26 °C. The temperature effect on the calibration line will be governed by the Nernstian equation and will give millivolt decade differences of 58·2 at 20 °C, 59·2 at 25 °C and 60·2 at 30 °C. The effect of temperature variation on the slope of an average calibration graph will therefore be marginal. It has been reported, however, that absolute measured potentials change[4] by 1·5 mV $°C^{-1}$ so that temperature changes occurring during measurement of standards or samples and the fixed concentration should be avoided and in no circumstances should exceed 1 °C.

INTERFERENCES—

The major ionic constituents of water, Na^+, K^+, Ca^{2+} and Mg^{2+}, added as chlorides, and CO_3^{2-}, HCO_3^-, NO_3^- and SO_4^{2-}, added as sodium salts, each at a 0·1 M concentration, had no significant effect on 10, 1·0 and 0·1 mg l^{-1} of ammoniacal nitrogen. Hydrazine, often added to water central heating systems, similarly had no effect when present at 1 mg l^{-1} levels.

Free chlorine necessarily interferes, owing to the presence of chloramines. Dechlorination was effected by standing the sample for 10 minutes with sodium sulphite (0·5 ml of a solution containing 1·8 g l^{-1}) or sodium thiosulphate (0·5 ml of a solution containing 7 g l^{-1}) prior to the addition of pH adjuster. Total dechlorination with sodium arsenite (0·5 ml of a solution containing 2 g l^{-1}) was unsuccessful.

DISTILLATION CONDITIONS—

Evaluation of the probe required comparison of the results obtained by using this method with those obtained by existing methods of ammonia determination. It has been reported that recovery of amounts exceeding 500 μg of ammoniacal nitrogen by distillation, for different pH conditions, is incomplete.[5] The recovery of ammonia, in 200 ml of distillate, from 0·5 g of sodium carbonate[2] and from alkaline permanganate solution[1] is shown in Table V. These values confirm that satisfactory recovery of ammonia is possible for levels not greater than 400 μg of ammoniacal nitrogen without distillation into dilute acid and, for the purpose of comparative values, aliquots of samples were distilled to ensure that the ammonia did not exceed this level. Thus, 500 ml of samples were distilled for levels less than 1·0 mg l^{-1} of ammoniacal nitrogen, 100 ml for levels in the range 1 to 4 mg l^{-1}, 25 ml for levels in the range 4 to 20 mg l^{-1} and 10-ml aliquots for levels exceeding 20 mg l^{-1}. These aliquots were diluted to 500 ml with de-ionised water and the blanks for each alkaline variant were measured daily when required.

TABLE V

RECOVERY OF AMMONIA BY DISTILLATION

Ammoniacal nitrogen added: A, 100; B, 200; C, 400; D, 1000; E, 4000 μg

	Ammoniacal nitrogen recovered, per cent.				
Distillation conditions	A	B	C	D	E
From sodium carbonate	100·8	98·3	100·0	99·9	97·4
From alkaline permanganate ..	100·0	100·5	100·6	96·6	95·0

It has been noted that interference may occur in spectrophotometric measurements using Nessler's reaction on ammonia distilled from water and wastewaters[1,3] and accordingly, all comparative values included, in addition to determination by Nessler's reaction, determination by the phenol - hypochlorite reaction (indophenol procedure). Of the many variations of the latter, an adaptation[6] was used that was based on the method of Weatherburn.[7]

METHOD

APPARATUS—

All glass apparatus should be stored containing de-ionised water.

Ammonia probe—This is an Electronic Instruments Ltd. Laboratory Model 8002–2. The probe is stored, when not in use and overnight, in 0·1 M ammonium chloride solution as directed. Before use, the probe should be kept for at least 30 minutes in a solution of 10 parts of 0·1 mg l^{-1} ammoniacal nitrogen and 1 part of alkaline EDTA pH adjuster, this solution being changed if there is a continuous drift in absolute potential. (For the experimental work described, when measurement was undertaken below 0·1 mg l^{-1} the probe was similarly kept in de-ionised water before use.)

pH or millivolt meter—Potentials are measured on a pH or millivolt meter capable of reading to 0·2 mV.

Magnetic stirrer—Solutions are stirred magnetically by using a polypropylene-covered bar magnet.

REAGENTS—

These should be of analytical-reagent grade.

Ammonia-free water—Water treated by passing it through a mixed resin bed is further purified by adding 5 g of a strong cation exchanger in the hydrogen form to 5 litres of this water and standing the mixture for 2 days.[3] The resulting water should not contain more than 2 μg l^{-1} of ammoniacal nitrogen as determined by distillation and spectrophotometric measurement.

pH adjuster—Dissolve 20 g of sodium hydroxide and 18·6 g of ethylenediaminetetraacetic acid dihydrate, disodium salt, in ammonia-free water and dilute the solution to 500 ml.

Sodium thiosulphate solutions, 0·7 per cent.—Prepare daily a solution containing 0·7 g of sodium thiosulphate pentahydrate dissolved in 100 ml of de-ionised water.

Standard ammonia solutions—Dissolve 3·821 g of ammonium chloride (dried at 100 °C) in de-ionised water in a 1-litre calibrated flask and dilute to 1 litre to give a solution containing 1000 mg l^{-1} of ammoniacal nitrogen. Dilute suitable aliquots with de-ionised water to give solutions containing 100, 40, 20, 10, 4 and 2 mg l^{-1}. Prepare daily, as required, solutions with concentrations of 1·0, 0·4, 0·2, 0·1, 0·04, 0·02 and 0·01 mg l^{-1} of ammoniacal nitrogen by dilution of suitable aliquots of the above standards with ammonia-free water.

PROCEDURE—

Transfer 50 $\pm$ 1 ml of a standard solution containing 1 mg l^{-1} of ammoniacal nitrogen into a Pyrex glass beaker and add 5 ml of pH adjuster immediately before measurement. Remove the existing sample, standard or the conditioning solution and dry the probe with a tissue. Immerse the probe in the standard solution, place the beaker on a magnetic stirrer and stir by using a small bar magnet. After 4 minutes measure the potential. Transfer 50 $\pm$ 1 ml of sample or distilled sample into a glass beaker, add 5 ml of pH adjuster, and similarly measure the potential after 4 minutes, or 8 minutes for levels equal to or less than 1·0 mg l^{-1} of ammoniacal nitrogen. Record the difference in potential between the sample and the reference standard concentration (1 mg l^{-1}) and read off the ammoniacal nitrogen concentrations from the calibration graph. For a series of samples, the reference standards can be repeated as necessary to correct for any drift in absolute potential.

If consecutive electrode readings involve a potential difference of greater than +60 mV, a second aliquot of the lower concentration sample must be taken and the reading repeated. If chlorine is present in a sample, add 0·5 ml of a freshly prepared sodium thiosulphate solution to 50 ml of the sample and stand the mixture for 10 minutes before addition of the pH adjuster.

The calibration graph for an individual probe should be prepared by using 50 ml of standard solutions in the range 0·01 to 100 mg l^{-1} of ammoniacal nitrogen, the potentials in both ascending and descending order of concentration being read. A graph of the logarithm of concentration *versus* the average difference in potential from that of the reference standard concentration (1 mg l^{-1}) is linear for the range 0·2 to 100 mg l^{-1}, and curves increasingly below the lower limit (Table IV). Allowance should be made for the ammonia level of the ammonia-free water at the lowest concentration levels in plotting this graph. When the calibration graph has been adequately defined for an individual probe, standard solutions need only be measured to ascertain the condition of the probe, or when the probe membrane has been changed.

TABLE VI

RECOVERY OF AMMONIA ADDED TO WATER AND WASTEWATERS
Ammonia added as nitrogen: A, 10; B, 1; C, 0·1 mg l^{-1}

Sample	Sample level as nitrogen/mg l^{-1}	Ammonia recovered as nitrogen/mg l^{-1} A	B	C
Spring water	<0·01	10·5	0·99	0·10
Well water	<0·01	10·0	0·99	0·10
Potable water, *ex* surface water	0·01	9·6	1·02	0·12
Borehole water	<0·01	10·3	1·01	0·10
Very hard well water	<0·01	10·1	1·01	0·11
Swimming-pool water	0·46	10·0	0·97	0·10
	0·04	9·9	1·01	0·11
Central heating system water	0·08	9·4	0·99	0·13
Lake water	0·01	10·2	0·99	0·10
	0·61	9·8	1·00	0·11
River water	0·37	10·0	0·99	0·11
Aged domestic sewage effluent	0·05	10·0	1·02	0·11
	0·07	9·9	1·05	0·11
	<0·01	9·9	1·01	0·07
Trade waste	0·72	9·7	1·01	0·07
	1·84	9·2	0·9	—
	0·62	9·6	0·96	0·11
Average recovery of nitrogen/mg l^{-1}		9·9	1·00	0·10
Coefficient of variation, per cent.		3·2	3·3	16·0

RESULTS

The recovery of ammonia was tested by addition of standard solutions to a number of samples from a variety of origins followed by immediate measurement with the probe. Two millilitres of solutions containing 500, 50 and 5 mg l^{-1} of ammoniacal nitrogen were diluted to 100 ml with sample to give added levels of 10, 1·0 and 0·1 mg l^{-1} of ammoniacal nitrogen. Allowance was made, when necessary, for the natural ammonia level to account for dilution of the water sample with standard. Swimming-pool water was pre-treated with sodium thiosulphate solution before measurement. The results are shown in Table VI. The average

TABLE VII

COMPARISON OF PROBE VALUES WITH VALUES FROM EXISTING METHODS FOR DETERMINATION OF AMMONIA

Sample	Nitrogen/mg l^{-1} Probe	Nessler's reaction	Indophenol method
Ammoniacal nitrogen—			
Potable water	0·05	0·07	0·05
Swimming-pool water	0·17	0·20	0·18
Potable water	0·24	0·22	0·22
Swimming-pool water	0·35	0·36	0·34
Ditch water	0·47	—	0·45
Raw borehole water	0·72	0·70	0·73
Domestic sewage effluent	1·3	1·3	1·2
Airfield drainage run-off	2·9	—	2·8
Domestic sewage effluent	5·2	5·7	5·3
Slaughterhouse waste	13	14	15
Partially treated sewage	25	27	27
Total ammoniacal and albuminoid nitrogen—			
Potable water	0·05	0·05	0·05
	0·09	0·09	0·09
Swimming-pool water	0·26	0·28	0·27
Potable water	0·45	0·43	0·47
Domestic sewage effluent	0·93	0·85	0·79
	9·0	9·3	9·1
Partially treated sewage	9·5	9·5	9·6
Trade waste	12	13	12
Partially treated sewage	21	22	21
Crude sewage	44	43	44

TABLE VIII

COMPARISON OF AMMONIACAL AND ALBUMINOID NITROGEN LEVELS IN WATER AND WASTEWATERS DETERMINED BY DIFFERENT METHODS

Sample	Ammoniacal nitrogen/mg l^{-1}			Albuminoid nitrogen/mg l^{-1}		
	Probe (direct)	Nessler's reaction and distillation	Indophenol reaction and distillation	Probe (by difference)	Nessler's reaction and distillation	Indophenol reaction and distillation
Potable water, *ex* surface water ..	0·03	0·03	0·03	0·19	0·19	0·18
Potable water	0·04	0·05	0·05	0·03	0·01	0·01
	0·06	0·06	0·06	0·03	0·01	0·01
Swimming-pool water	0·21	0·22	0·21	0·05	0·08	0·07
	0·21	0·21	0·19	0·29	0·23	0·22
Domestic sewage effluent	0·53	0·53	0·53	0·9	0·8	0·9
	1·7	2·0	1·7	0·7	1·0	1·0
	7·2	7·1	6·6	1·4	1·8	1·6
Partially treated sewage	8·4	8·0	8·9	0·9	0·9	1·0
	13	12	12	0·5	0·7	0·7
	25	26	25	2·6	2·6	2·6
Crude sewage	43	41	42	0·5	2·1	2·2

recovery at each level was satisfactory, the coefficient of variation being 3·3 per cent. at the 1 and 10 mg l^{-1} levels of added ammoniacal nitrogen and 16 per cent. at the 0·1 mg l^{-1} concentration. These values are in good agreement with the precision for electrode calibration.

Free ammonia levels obtained by direct measurement with the ammonia probe and values obtained by distillation from 0·5 g of sodium carbonate, with subsequent spectrophotometric measurement by use of Nessler's reaction or the indophenol method, are illustrated for a variety of samples in Table VII. Also included is a comparison of total ammoniacal and albuminoid nitrogen obtained by distillation from alkaline permanganate, and determination both with the probe and by spectrophotometry. Because of the transient nature of ammoniacal nitrogen, all determinations were made at the same time. The satisfactory agreement obtained in these trials suggested a more stringent comparative exercise. This involved direct measurement of ammoniacal nitrogen with the probe and measurement of the total ammoniacal and albuminoid nitrogen with the probe after distillation, thus obtaining the albuminoid nitrogen level by difference. The values were compared with values obtained by consecutive distillation of the ammoniacal and albuminoid nitrogen and determination by spectrophotometric measurement; the results are shown in Table VIII. The agreement, however, may be affected by the precision of the electrode, which can introduce disproportionate errors into the albuminoid nitrogen concentrations when the ratio of free to albuminoid nitrogen is large.

Conclusions

The ammonia probe can be used in laboratory conditions to measure the discrete free and saline ammoniacal nitrogen present in a wide range of water samples. These include swimming-pool waters, surface waters, effluents, sewages and wastewaters. Determination should be possible within a precision of 4 per cent. for ammoniacal nitrogen levels greater than 0·4 mg l^{-1}, and within 0·015 mg l^{-1} for levels less than 0·4 mg l^{-1}. Samples can be measured in random order providing consecutive readings involve absolute potential differences of not more than +60 mV. The interference most likely to be encountered is chlorine as chloramines, but dechlorination is readily achieved with thiosulphate. The calculated lower limit of detection of 0·03 mg l^{-1} of ammoniacal nitrogen and the precision at low levels (less than 0·1 mg l^{-1}), which originates partly from the non-Nernstian response at these levels, suggest that its accurate determination in potable waters would be difficult.

The probe can also be used to determine albuminoid nitrogen, subject to the precision of the probe being acceptable, by taking the difference between the ammoniacal nitrogen and the total free ammoniacal *plus* albuminoid nitrogen obtained after distillation from alkaline permanganate.

We thank Mrs. Alison Hamilton-Sharp for some technical assistance. This paper is published by permission of the Government Chemist.

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Received *November 9th*, 1973
Accepted *December 28th*, 1973

Analyst, June, 1974, Vol. 99, *pp.* 376–380

The Coulometric Determination of Trace Levels of Sulphur in Gallium Phosphide

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Sulphur present in gallium phosphide has been determined. At 950 °C gallium phosphide reacts with platinum and hydrogen to form Ga_xPt_y and platinum phosphide (PtP_2), and sulphur is catalytically converted into hydrogen sulphide. The latter is absorbed in an alkaline medium and determined by controlled-potential coulometry at a silver-gauze electrode. The determination of 0·05 μg of sulphur is shown to be possible, the blank value by this method amounting to 0·01 μg of sulphur.

Sulphur present in gallium phosphide has in the past been determined by Luke's method.[1] Because of the high blank value of the reagents, which amounts to 1 or 2 μg of sulphur,[2] this method is not very suitable for determining small amounts of sulphur.

Starting from the sensitive and reproducible sulphide determination by controlled-potential coulometry with a silver electrode ($2\ Ag + S^{2-} \rightarrow Ag_2S + 2e$),[3] we investigated the possibility of converting the sulphur present in gallium phosphide into hydrogen sulphide. It is known from the literature[4,5] that the sulphur in both organic and inorganic sulphur-containing compounds can be converted into hydrogen sulphide by reduction with hydrogen on a platinum catalyst at elevated temperatures.

Experimental

Reagents—

Quartz-distilled water was used throughout this work.

Sodium hydroxide solution, 0·1 M—This solution is prepared from analytical-reagent grade sodium hydroxide.

Hydrogen—Hydrogen is purified by passing it through a liquid nitrogen cold trap so as to remove any water present.

Nitrogen—Nitrogen is purified by passing it through a copper furnace and a column filled with soda-lime in order to remove organic compounds and oxygen.

Calibration solutions—

The following solutions of sulphur-containing compounds were prepared.

Sulphosalicylic acid solution—This solution is prepared by dissolving 80·0 mg of sulphosalicylic acid in 100 ml of water.

Potassium sulphate solution—This solution is prepared by dissolving 54·5 mg of potassium sulphate in 250 ml of water.

Cell—

The cell used for the determination of hydrogen sulphide is shown in Fig. 1. The working electrode is a silver-gauze electrode with an apparent surface of 30 cm^2, which is kept at a constant potential of −360 mV *versus* a saturated calomel electrode, and the auxiliary electrode is a platinum foil immersed in a 1 M potassium nitrate solution. Contact with the 0·1 M sodium hydroxide solution is made via a conducting agar bridge. The potential of the working electrode is controlled by a Wenking potentiostat. The current resulting from the reaction of sulphide at the silver electrode is passed through a standard resistor (1000 Ω), the voltage drop across the resistor being converted into a frequency (Anadex DF-100), which is measured with an electronic counter (Philips PM6620). The signal is corrected for the residual current due to the reaction of contaminants, and the current integration system electrically calibrated with a constant-current source.

PLATINUM CATALYST—

Quartz-wool or hexagonal silicon carbide crystals (size 3 mm) are wetted with a chloroplatinate solution containing 2·5 g of platinum per 100 ml, dried and subsequently heated in order to decompose the chloroplatinate and to form finely divided platinum metal at the surface of the substrate. The catalyst is then heated at 1000 °C in an atmosphere of hydrogen for 1 day so as to remove contaminants (*e.g.*, halides). Silicon carbide crystals are to be preferred as supporting material because they can be packed in a more reproducible way than quartz-wool.

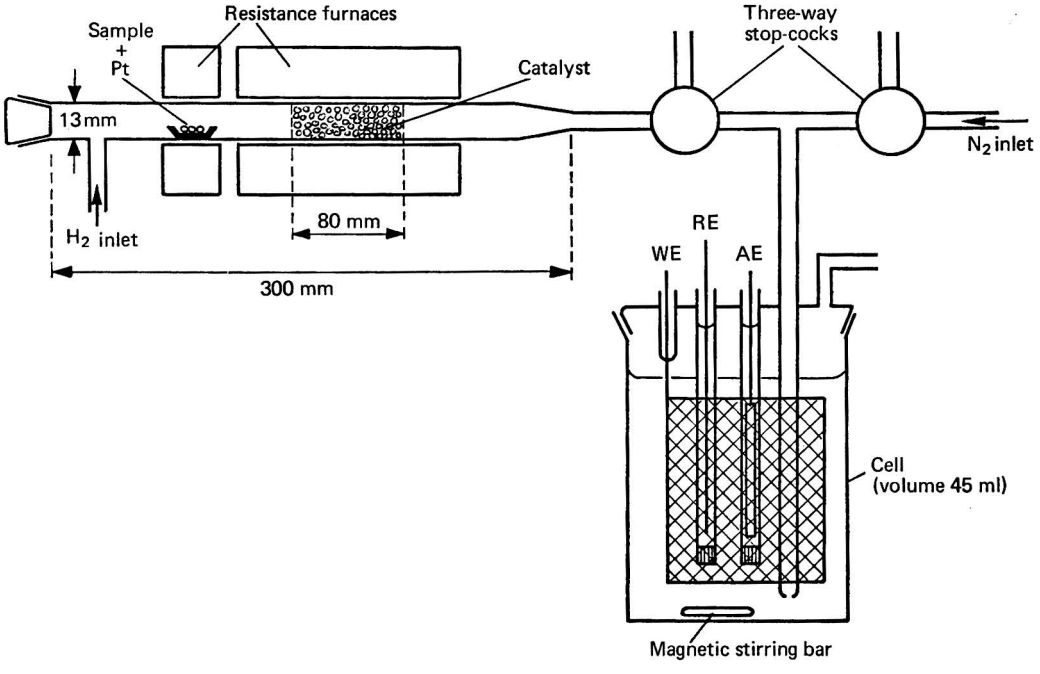

Fig. 1. Experimental set-up for the determination of sulphur in gallium phosphide. Electrodes: WE, working; AE, auxiliary; and RE, reference

PROCEDURE—

The gallium phosphide sample is wrapped in a platinum foil and the latter placed in a quartz crucible, which is then inserted into the quartz tube (see Fig. 1). At the beginning of the procedure the sample is not heated. Purified hydrogen is led through the tube while the catalyst is heated at 900 °C; this hydrogen is not led into the electrolysis cell. Meanwhile, purified nitrogen is led into the cell in order to remove oxygen. The presence of oxygen in the cell causes a cathodic current to flow (due to reduction of the oxygen). The flow of nitrogen is continued until the cathodic current has diminished to a value below 0·05 μA, which indicates that oxygen has been removed. The nitrogen flow is then stopped and the hydrogen, which is first passed over the heated platinum catalyst, is led into the electrolysis cell. (Care must be taken to prevent the solution from entering the inlet tube.)

When the anodic current has fallen to a value below 0·05 μA (indicating the removal of contaminants), the sample in the tube is heated at 950 °C with the second resistance furnace. Gallium phosphide reacts with platinum and hydrogen while the sulphur is released partly as hydrogen sulphide. However, in order to achieve the complete conversion of the sulphur into hydrogen sulphide it is necessary to use a platinum catalyst.

WORKING CONDITIONS FOR THE CATALYST—

The conversion of the sulphur present in sulphosalicylic acid into hydrogen sulphide was carried out in order to check the method and to determine the optimum working conditions; 20 μl of the sulphosalicylic acid solution (corresponding to 2·0 μg of sulphur) were evaporated to dryness in a platinum crucible, which was brought into the quartz tube and the acid vaporised at 950 °C. With the hydrogen flowing at the rate of 10 ml min^{-1}, the vapour was led over the platinum catalyst. The experiment was repeated several times with the platinum catalyst at different temperatures. The results obtained are shown in Fig. 2, in which the integrated current due to the reaction of sulphide at the silver electrode is shown as a function of the heating time at different temperatures of the catalyst. Different hydrogen flow-rates were also tried, the temperature of the catalyst being maintained at 900 °C. From these experiments, the following optimum working conditions were determined: a hydrogen flow-rate of 10 ml min^{-1} and a catalyst temperature of 900 °C.

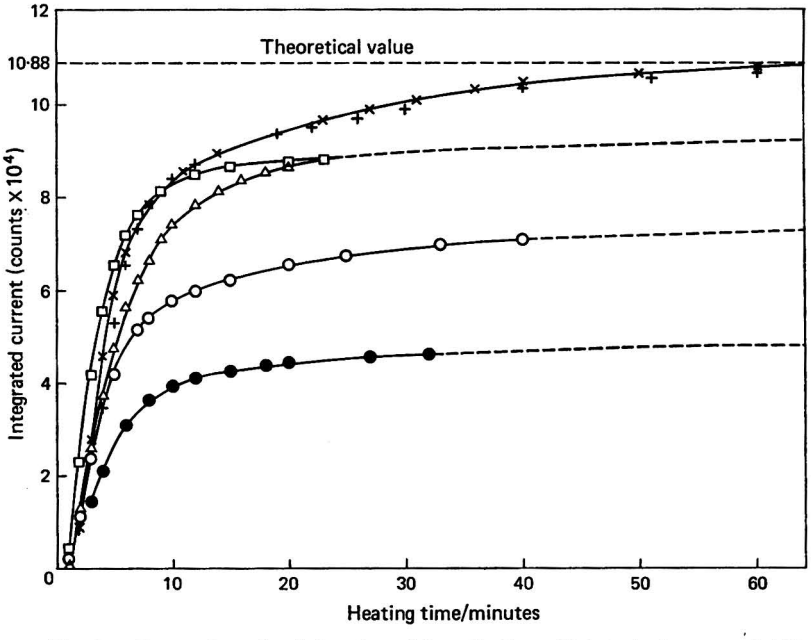

Fig. 2. Conversion of sulphur in sulphosalicylic acid into hydrogen sulphide on a platinum catalyst as a function of time and at different temperatures of the catalyst. Hydrogen flow-rate of 10 ml min^{-1}. Temperature/°C: ●, 500; ○, 600; △, 700; □, 800; ×, 900; and +, 1000

Sulphur in inorganic compounds, *e.g.*, potassium sulphate, can be determined in the same way, the optimum working conditions being identical with those for determining sulphur in organic compounds. The nature of the substrate of the catalyst has no influence on these conditions.

RESULTS AND DISCUSSION

CONVERSION YIELD—

The yield from the conversion of sulphur into hydrogen sulphide is calculated by comparing the integrated current, due to the reaction of sulphide at the silver electrode, with a calibration graph; the latter is prepared by plotting the integrated current, from a coulometer or from a constant-current source, *versus* the amount of sulphide, which is calculated according to Faraday's law with $n = 2$. Amounts of sulphosalicylic acid and potassium sulphate corresponding to 2·0 μg of sulphur were analysed for sulphur according to the procedure described above. The results obtained are presented in Table I.

TABLE I

DETERMINATION OF SULPHUR IN SULPHUR-CONTAINING COMPOUNDS (EQUIVALENT TO 2·0 μg OF SULPHUR)

Experimental conditions: hydrogen flow-rate 10 ml min^{-1}; sample heating temperature, 950 °C; catalyst heating temperature, 900 °C; and time of analysis, 1 hour

Sulphosalicylic acid found, per cent.	Potassium sulphate found, per cent.
94	93
97	99
103	92
97	95
96	97
94	98
Mean 97 ± 3 (1σ)	Mean 96 ± 3 (1σ)

The above results lead to the conclusion that the yield from the conversion of sulphur into hydrogen sulphide under the given experimental conditions is satisfactory for both types of material and that sulphur in gallium phosphide may also be expected to be converted into hydrogen sulphide, especially as the gallium phosphide is decomposed during the reaction, as described below.

DETERMINATION OF SULPHUR IN GALLIUM PHOSPHIDE—

Gallium phosphide reacts with platinum at 950 °C in an atmosphere of hydrogen. From X-ray diffraction analysis the reaction products were shown to be a mixture of a gallium - platinum alloy (Ga_xPt_y) and platinum phosphide (PtP_2). Sulphur present in gallium phosphide is released, converted into hydrogen sulphide on the platinum catalyst and determined coulometrically as sulphide in the electrolysis cell.

Because of the reaction between gallium phosphide and platinum, the sulphur can be determined without interference from either gallium or phosphorus. This conclusion was checked by analysing gallium phosphide crystals* that were heavily doped with sulphur (98 p.p.m.), which were allowed to react with different amounts of platinum. The reaction was followed by monitoring the hydrogen sulphide formed. The results obtained are presented in Table II.

TABLE II

REACTION OF GALLIUM PHOSPHIDE WITH PLATINUM

Amount of gallium phosphide taken/mg	Amount of platinum used/mg	Sulphur found, p.p.m.	Analysis time/hours
20	130	95	2
21	270	96	1
20	340	99	1

In order to obtain a rapid and quantitative reaction, a sufficient amount of platinum is required, irrespective of the physical nature of the gallium phosphide samples. For the determination of sulphur in gallium phosphide we used 300 mg of platinum per 20 mg of sample. The release of sulphur is not the rate-determining step because the analysis time is 1 hour for potassium sulphate and sulphosalicylic acid as well as for gallium phosphide. For comparison, gallium phosphide crystals from the same samples were analysed by Luke's method. The results for both methods are shown in Table III.

The results obtained by the two methods correspond very well, the striking difference being that with the coulometric method very small amounts of material can be analysed; down to 100 p.p.m. of sulphur in 0·55 mg of gallium phosphide (≡0·05 μg of sulphur) can be determined by the coulometric method.

Luke's method is not suitable for determining small amounts of sulphur because of the high blank value (1 to 2 μg of sulphur),[2] which results from contamination by the chemicals

*Prepared in our laboratory.

used, either from the atmosphere or from the analyst, or both, and attempts to eliminate these sources of contamination make the method more time consuming.

TABLE III

COMPARISON OF RESULTS OBTAINED BY LUKE'S METHOD AND THE COULOMETRIC METHOD

Luke's method[1]		Coulometric method	
Amount of gallium phosphide taken/mg	Sulphur found, p.p.m.	Amount of gallium phosphide taken/mg	Sulphur found, p.p.m.
150	104	20·94	100
150	98	10·85	102
150	99	5·43	94
150	100	2·89	101
	Mean 100	0·55	102

The advantage of the coulometric method is that it gives a low blank value (about 0·01 μg of sulphur) as the reactants are hydrogen and platinum metal, which can be purified very easily. Also, the method is carried out in a closed system, which reduces the risk of contamination. The coulometric method is also sensitive, as shown in Table III, as down to 0·05 μg of sulphur can be determined by this method, which is not possible by Luke's method. With the method described above, sulphur can be determined in gallium arsenide (GaAs) and gallium arsenide - phosphide ($GaAs_xP_{1-x}$).

CONCLUSION

Gallium phosphide reacts with platinum at 950 °C in an atmosphere of hydrogen to form Ga_xPt_y and platinum phosphide, while sulphur present in the gallium phosphide is quantitatively converted into hydrogen sulphide on a platinum catalyst. Measurement, by controlled-potential coulometry, of the hydrogen sulphide thus formed offers a sensitive method for the determination of sulphur in gallium phosphide.

The reagents (platinum and hydrogen) can be purified easily and efficiently, resulting in a very low blank value (0·01 μg of sulphur) as compared with Luke's method (1 to 2 μg of sulphur) and 0·05 μg of sulphur in gallium phosphide has been determined by this method.

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Received *May* 21st, 1973
Amended *November 8th*, 1973
Accepted *December 14th*, 1973

Book Reviews

LIQUID SCINTILLATION COUNTING. Volume 2. PROCEEDINGS OF A SYMPOSIUM ON LIQUID SCINTILLATION COUNTING ORGANIZED BY THE SOCIETY FOR ANALYTICAL CHEMISTRY, BRIGHTON, ENGLAND, SEPTEMBER 13–16, 1971. Edited by M. A. CROOK, P. JOHNSON and B. SCALES. Pp. xii + 327. London, New York and Rheine: Heyden & Son Ltd. 1972. Price £8·75; $21.50; DM72.

This book is the second volume of a series, the first one reporting a Symposium held at Salford in 1970. It is divided into five sections corresponding to the sessions of the Symposium and it contains five plenary lectures and twenty-two shorter papers. The first chapter, by J. B. Birks and G. C. Poullis, gives an assessment of scintillator solution materials under conditions close to those used for current internal liquid scintillation counting. Solvents, primary solutes and the effect of quenching on them, and secondary solutes are assessed on the basis of relative pulse heights determined by a channels ratio method with carbon-14 as an internal source. Nearly twenty years ago a large number of compounds were synthesised and tested at Los Alamos, the pulse heights being measured by using an external radiation source. It is interesting to see how little the over-all picture has changed. This section of the book includes chapters on the absolute counting of beta-emitters, the use of an approximate mathematical model to explain the different effects of chemical and colour quenching on pulse-height distributions and a scintillation detector for labelled materials in high-pressure liquid chromatography.

The second section of the book opens with a historical account of the development of the modern scintillation counter by E. Rapkin. Due to the wide use of liquid scintillation counting techniques in biochemistry and medicine the development of equipment has been mainly carried out by commercial instrument manufacturers, and a high level of sophistication has been reached. It is a competitive business. A firm that was technically successful in producing significant advances disappeared from the scene, leaving its successes to posterity in the instruments of its commercial competitors. The section continues with an account of problems experienced in chemiluminescence and how they were solved, or else remain mysteries, and concludes with two chapters on the bioluminescence assay of adenosine triphosphate and related compounds. This technique is included with scintillation counting because the equipment is suitable for the measurement of low light intensities, although it is not "counting" in the accepted sense, and no radioactivity is involved. However, "a fig for systems," as J. H. Fabre said when he included spiders in a book on insects.

The third section is concerned with sample preparation for counting inorganic materials and the opening chapter by A. Dyer adequately reviews the field. The chapters that follow deal with the counting of calcium-45 in biological samples in the presence of strontium-90, the determination of low levels of alpha-emitting plutonium isotopes and plutonium-241 (a low-energy beta-emitter) in urine by gel scintillation counting and the counting of carbon-14 and phosphorus-32 tracers in work on biological processes in polluted waters. This section includes a paper on radiocarbon dating with special reference to the use of liquid scintillation counting. The final chapter of the section describes a continuous automatic system for the Cerenkov counting of some fission nuclides by using substoicheiometric solvent extraction and displacement through ion-exchange resins.

The subject of the fourth section is sample preparation of organic materials. B. W. Fox describes sample preparation techniques in biochemistry with special emphasis on emulsion counting. The need for a full understanding of the fundamental processes involved in these emulsion systems, in order to avoid erroneous results, is emphasised in the discussion on the paper. The author of the paper deprecates the use of commercial "cocktails" based on secret recipes, which make the choosing of appropriate methods of quench correction in liquid scintillation counting impossible. The problems arising in heterogeneous counting are also evident in subsequent papers in the section, which deal with the counting of labelled biological macromolecules and plasma samples. One speaker emphasised that counting after combustion was the best way to avoid these difficulties although there is no paper dealing specifically with this subject. It is to be hoped that it will be included in a later volume.

The final section of the book is on data processing. An introductory paper by J. L. Spratt reviews the problems of data acquisition and the considerations that should decide the choice of computing facility. He suggests that excellent data handling is often performed on inappropriate data leading to "beautifully typed sheets essentially ready for publication—except that the

figures are nonsense." The next paper gives a description of an off-line computer programme for processing multilabel counting data with three nuclides. It includes the calculation of errors associated with external standard channels ratio quench correction and the effect on the former of irregular vial geometry. Other papers include one on drug distribution studies, in which the counting data are processed off-line to give the final pharmacokinetic constants required, and one on the computer handling of data for the radioimmunoassay of insulin labelled with iodine-125. The final paper gives a detailed account of the use of an off-line computer for processing data in connection with the applications of tritium tracers to problems in the oil industry.

While this book, being one of a series, does not cover the subject completely, the papers and the discussions that follow them give much practical detail which should be particularly useful to those using this form of counting as a means to an end. The book is clearly set out and well produced. The comment one could make on price is the usual one today. D. I. COOMBER

FUNDAMENTAL ASPECTS AND RECENT DEVELOPMENTS IN OPTICAL ROTATORY DISPERSION AND CIRCULAR DICHROISM. PROCEEDINGS OF NATO ADVANCED STUDY INSTITUTE HELD AT TIRRENIA (PISA), 5–18 SEPTEMBER 1971. Edited by F. CIARDELLI and P. SALVADORI. Pp. xviii + 419. London, New York and Rheine: Heyden & Sons Ltd. 1973. Price £11; $30.25; DM90.

This book constitutes the published proceedings of the conference held under the auspices of NATO at Tirrenia, near Pisa, in 1971. The contributions included are only those of the invited speakers; they do, however, cover a wide range of work. The theoretical side of the study of optical activity is represented by papers from Mason, Rosenfeld and Moscovitz, Weigang and Tinoco and colleagues, while papers on the more empirical aspects of stereochemistry include a contribution from Kirk, Klyne, Scopes, Snatzke and Blout. There is an absence of studies in the carbohydrate field. Solvent effects are discussed by Legrand.

Obviously, this is a useful sort of book for those who are *aficionados* but who were not able to spend a fortnight on the Italian littoral. Those not previously acquainted with the subject will, however, be able to pick up enough basic material from the introductory chapter(s) to attempt an understanding of the research papers. It is, in fact, refreshing to find so much historical material in the book, since it will help to counteract the impression that optical rotatory dispersion (ORD) and circular dichroism (CD) are phenomena discovered in America in the last 20 years. The book is made up of parts, so that the great names of the past, such as Arago, Biot, Fresnel, Cotton, Lowry and Kuhn, do not stand out with the clarity that would have resulted from a chapter devoted solely to the historical aspects.

Because the book is a record of papers given at the conference, suitably edited, it does not include the discussions, which are often the most useful part of a conference from the point of view of the participants. On the other hand, there are contributions by many of the well known practitioners in the field, covering the subjects that have been most studied, *i.e.*, the carbonyl group, the carboxyl group and co-ordination compounds.

Out of line with the rest of the book in practical terms is the section on MCD. Although MORD and MCD measurements require modification of normal ORD or CD apparatus, the effect of these modifications is to remove the main restriction on this area of work, *i.e.*, the need to have optically active substances to work with. Those who were prepared to resolve compounds in order to prove stereochemical points have always been a rather select band. In the study of ORD and CD they were joined by others whose interest lay in the field of optically active natural products. The techniques of MORD and MCD bring the study of optical activity within the orbit of spectroscopists generally, as was shown by the attendance at the Faraday Society symposium on "Magneto Optical Effects" in 1970. Thus, whilst CD studies are useful in elucidating the structure and origin of ultraviolet absorption, MCD is a technique that is likely to be developed mainly by spectroscopists in order to obtain a better understanding of ultraviolet spectra in general, rather than by stereochemists as an extension of their field.

So far as analytical applications are concerned, it is wise to be aware that the new techniques have not, in general, improved in accuracy on the time-honoured visual polarimeter, although with photoelectric instruments a reading can be obtained with much less material than is required for a visual instrument. The use of ORD and CD techniques for stereochemical correlation has analytical implications and the MCD technique, by helping in the understanding of ultraviolet spectroscopy, will influence analysis indirectly, and in particular cases it clearly could be used as a direct analytical technique if cheaper methods should fail. M. K. HARGREAVES

SUGAR CONFECTIONERY AND CHOCOLATE MANUFACTURE. By R. LEES and E. B. JACKSON. Pp. xx + 379. Aylesbury: Leonard Hill Books. 1973. Price £8·50.

The number of books in the field of sugar and chocolate confectionery is not as great as it might be, so that a contribution in this field is to be welcomed.

The book, comprising some 380 pages, is set out logically with an introductory chapter dealing with basic technical considerations leading to several chapters detailing the characteristics of the more usual ingredients. The latter two thirds of the book are concerned mainly with the manufacture of cocoa, chocolate and related products; boiled sweets, caramels, toffees and fudges; fondants, creams and crystallised confectionery; gums, jellies and pastilles; liquorice and cream paste; tablets, lozenges and extruded paste; marshmallow and nougat; and other types of confectionery. One chapter includes information on the calculation of recipes from analytical results and a collection of reference tables is included. The section covering the various types of sugar and related materials could, perhaps, with advantage have included molasses, and the possible use of sorbitol in diabetic formulations could have been mentioned as fructose is discussed in this context.

Unfortunately, there appear to be one or two errors in the chapter on cocoa beans, but the section on fats and related ingredients provides a brief but useful description of several fats, antioxidants and surface-active agents commonly considered for use in confectionery production; that on milk and milk products deals with milk, condensed milk, condensed whey, dried milk powders, sodium caseinate, lactose and butter. Gelling, whipping agents and gums are covered separately. The chapter on flavouring and colouring agents additionally includes a great deal of summary information on various nuts, dried fruit and other additives, which, it could be argued, merit chapters of their own.

Readers may not find the chapter on cocoa, chocolate and related products entirely logical in its layout, and the order of the various sub-sections could be improved. For example, the sections on chocolate recipes and milk crumb have for some reason been interposed between those dealing with cocoa bean roasting and cocoa bean winnowing. Useful charts of possible faults and their prevention are included for many of the types of confectionery considered and there are a number of references quoted throughout, should more information be required on particular topics.

While a section is included on the calculation of recipes from analytical results, no analytical methods are included in the book, and greater emphasis could, with advantage, have been devoted to aspects of quality control, including hygiene, microbiological considerations and, possibly, legislation.

Not all manufacturers will find themselves in complete agreement with some of the views expressed, but nevertheless the book should prove to be a useful addition to the relatively few available and be of particular interest to newcomers in the field. P. H. WIGGALL

INSTRUMENTAL METHODS OF FOOD ANALYSIS. By A. J. MACLEOD. Pp. vi + 802. London: Elek Science. 1973. Price £12.

The majority of books on this topic emanate from the U.S.A., so that this work by Dr. MacLeod is to be welcomed.

This is an intriguing book and in many aspects original in its approach and the result of much hard work. There is no Preface, but the Introduction commences with a consideration of why foodstuffs are analysed and then discusses instrumental methods *versus* non-instrumental methods, an argument which I believe is a *non sequitur*, but I must criticise the statement that "legal requirements demand the use of traditional methods" and that "instrumental methods are legally inadmissible as evidence in a court of law." This statement is misleading since the number of "official" methods in the U.K. is minute and the official analyst has the discretion to use any method of value which will enable an accurate result to be obtained. All Public Analysts' laboratories have ultraviolet - visible spectrophotometers and gas chromatography facilities and most have at least a selection of atomic-absorption, infrared, polarographic and electrophoresis instrumentation available.

The discussion on sampling and extraction in Chapter 2 is adequate to give the reader an insight into the difficulties involved. The theoretical aspects of paper, thin-layer, ion-exchange, gel-permeation, column and electro-chromatography are dealt with in great detail, followed by a discussion of their applications to a wide range of foodstuffs. The tables on the use of the named techniques for determining various components are useful and valuable, followed by a selected bibliography and literature references, which unfortunately do not go beyond 1970. In similar

style, gas chromatography and ultraviolet and visible spectrophotometry are dealt with in very great detail and the above techniques comprise about two thirds of the work.

I am rather surprised that atomic-absorption spectrophotometry receives such scanty treatment, since, as with all instrumental techniques, the instrument makers state that it will solve all our problems without too much effort—until we come across the snags in practice. The underrated use of polarography in food analysis is given further emphasis by the author's treatment, no mention being made of derivative or a.c. pulse techniques; short descriptions of infrared, nuclear magnetic resonance and mass spectrometry and an outline of automated analysis follow. Polarimetry and refractometry are considered to be out of date, although fairly short descriptions are given; a visit to food factories would soon alter the author's opinion of their usefulness in a routine food laboratory.

In my opinion the writing is rather loose; for example, the headings Results and Interpretation could be more correctly described as the Interpretation of Instrumental Data. Also, when discussing the determination of caffeine by ultraviolet spectroscopy, the interference of 5-hydroxymethylfurfuraldehyde is described (page 471) "which is not found in tea but found in coffee and thought to originate in chicory"! Again, further down the same page, consideration is given to the simultaneous determination of benzoic acid and methyl *p*-hydroxybenzoate; mention should be made of the possible presence of sorbic acid, which has a maximum absorption very near to the latter and has been found together with the other two preservatives in some processed foodstuffs.

An example of the uncritical approach to the literature quoted is that on page 310, in which the methyl esters of the minor fatty acids are quoted as a means of establishing the authenticity of pork products, but no mention is made that pigs may be fed on beef offals and thus, if minor fatty acids are present, the original meat can still be genuine pork meat.

There are very few misprints and errors, the most serious is a "not" omitted on page 313 under analysis of pesticides and other residues in food by gas chromatography, otherwise it would appear that all possible agricultural chemical residues are being considered. A random check on literature references did not reveal any errors.

On the front inside dust cover, it is stated that it is a textbook for all students taking courses in food analysis or food science. I beg to disagree—the scope and theoretical nature are, in my experience, too advanced for that group, but it can be recommended as a reference book for official and consultant laboratories, together with industrial laboratories who would like to have a book that will introduce instrumentation to newcomers and constitute a ready means of finding out how to determine a constituent of food without having to look up too many references.

S. LANDSMAN

Erratum

JANUARY (1974) ISSUE, p.70, line 15. *For* "aminohydrazone" *read* "amidinohydrazone"

APPOINTMENTS VACANT

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Ionic Polymerisation as a Means of End-point Indication in Non-aqueous Thermometric Titrimetry Part VI. The Determination of Thiols

Alkyl and aryl thiols have been determined in the presence of carboxylic acids and phenols by means of acid - base catalytic thermometric titrimetry. Two titrations are carried out, with acrylonitrile and acetone as the end-point indicators. With the former indicator, thiol groups are not determined, so that the difference between the titration values obtained by using the two methods of end-point indication is a measure of the thiol content.

The thiol content of 2-mercaptothiazoline, 4,6-dihydroxypyrimidine-2-thiol (2-thiobarbituric acid), purine-6-thiol and 2-mercaptobenzimidazole can be determined by the same procedure. In the titration of 2-thiohydantoin, 4-hydroxypyrimidine-2-thiol (2-thiouracil), 2-mercaptobenzoxazole and 2-mercaptobenzothiazole, however, both end-point methods give the same titration value. These apparently anomalous results can be explained if it is accepted that the last four heterocyclic thiols exist in the thione tautomeric form in dimethylformamide solution. Some thioamides also titrate as acids, and differences between titration values obtained by using the two methods of end-point indication can again be attributed to thione - thiol tautomerism.

Thiols can be determined conveniently in amounts down to 0·01 mequiv, *i.e.*, about 2 mg of dodecane-1-thiol, with 0·1 M titrants. In instances when the acrylonitrile method can be used for the direct determination of the thiol function, 0·001 M titrant can be used and the lower level of determination is then about 0·0001 mequiv.

E. J. GREENHOW and Miss L. H. LOO

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

Analyst, 1974, **99**, 360–366.

Determination of Ammonia Levels in Water and Wastewater With an Ammonia Probe

The application of an ammonia probe has been investigated for discrete laboratory measurement of ammonia levels in a variety of waters. The probe displays a Nernstian response for the range 0·2 to 40 mg l^{-1} of ammoniacal nitrogen in a stirred 0·1 M sodium hydroxide solution containing 0·01 M ethylenediaminetetraacetic acid. Recoveries of added ammonia from a wide range of water samples are satisfactory. Both these recoveries of added ammonia and repeated calibrations of the probe suggest a precision of 4 per cent. for ammoniacal nitrogen concentrations greater than 0·4 mg l^{-1} and 0·015 mg l^{-1} for concentrations less than 0·4 mg l^{-1}; the statistical limit of detection is 0·03 mg l^{-1}. Good agreement is obtained with existing methods based on distillation and spectrophotometric measurement for a further range of samples, but the limit of detection and the precision at low levels suggest that accurate determination in potable waters would be difficult.

The probe can also be used to determine albuminoid nitrogen by taking the difference between the ammoniacal nitrogen and the total free *plus* albuminoid nitrogen obtained by distillation. Values obtained in this way agree with those obtained by existing methods subject to the precision of the probe being acceptable.

W. H. EVANS and B. F. PARTRIDGE

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Analyst, 1974, **99**, 367–375.

The Coulometric Determination of Trace Levels of Sulphur in Gallium Phosphide

Sulphur present in gallium phosphide has been determined. At 950 °C gallium phosphide reacts with platinum and hydrogen to form Ga_xPt_y and platinum phosphide (PtP_2), and sulphur is catalytically converted into hydrogen sulphide. The latter is absorbed in an alkaline medium and determined by controlled-potential coulometry at a silver-gauze electrode. The determination of 0·05 μg of sulphur is shown to be possible, the blank value by this method amounting to 0·01 μg of sulphur.

K. GIJSBERS, L. BASTINGS and R. van de LEEST

Philips Research Laboratories, Eindhoven, The Netherlands.

Analyst, 1974, **99**, 376–380.

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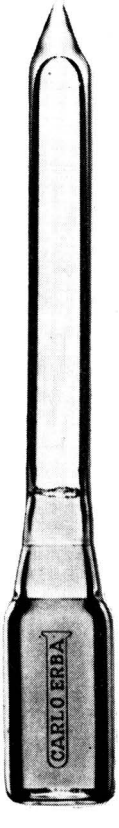
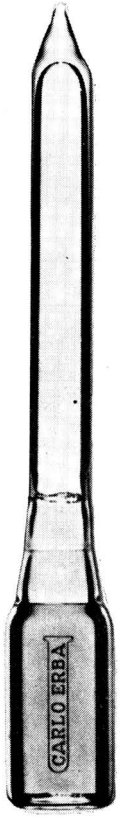


Hydrocarbons

This list includes all the normal chain paraffinic hydrocarbons from C4 to C40 and the principal isomers from C4 to C9, as well as some of the main cycloparaffinic, olefinic, diolefinic, cycloolefinic and aromatic hydrocarbons.

Fatty Acids

This list includes all the saturated fatty acids from C4 to C24, C9 pelargonic acid and the principal C16, C18, C22 unsaturated acids. All these products are straight-chain compounds.



Methyl esters of Fatty Acids

This list includes the Methyl esters of the above mentioned Fatty acids, as well as the C3, C5, C7.

Alcohols

This list includes the normal C4 and C5 Alcohols, together with their secondary, tertiary and branched chain isomers; moreover the normal C6, C7, C8, C9, C10, C11, C12.

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Via Carlo Imbonati 24
1-20159 Milano

Volume 99, No. 1179, Pages 313-384 June, 1974

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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Printed for the Society for Analytical Chemistry by Heffers Printers Ltd., Cambridge, England
All communications to be addressed to the Managing Editor, The Analyst, 9/10 Savile Row, London, W1X 1AF
Enquiries about advertisements should be addressed to J. Arthur Cook, 9 Lloyd Square, London, WC1X 9BA

Entered as Second Class at New York, U.S.A., Post Office