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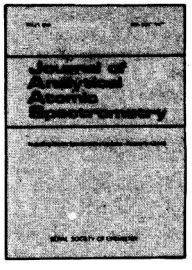

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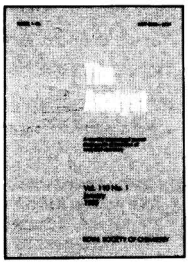

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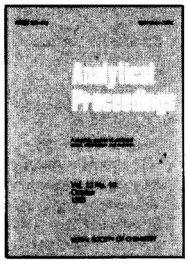

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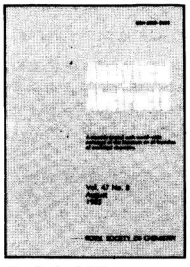

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Analytical Applications of the Catalysed Iodine - Azide Reaction A Review

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Summary of Contents

Keywords: *Review; iodine - azide reaction; kinetic methods*

Introduction

In acidic or neutral media, a solution of sodium azide and iodine remains practically unchanged for a long time, but in the presence of divalent sulphur compounds the irreversible redox reaction

$$2N_3^- + I_2 \rightarrow 2I^- + 3N_2$$

proceeds rapidly, giving rise to bleaching of the solution and the evolution of nitrogen bubbles.

This reaction was described by Raschig in 1904,[1] and has since then been the object of numerous studies. In addition, many analytical procedures have been developed that allow the sensitive and selective identification and determination of the compounds that catalyse the reaction.

In this paper, the analytical applications of the iodine - azide reaction are reviewed, and the diverse significant aspects involved are critically examined, together with the methods that have been used to follow the reaction and the analytical characteristics of the procedures proposed.

Mechanism of Reaction and Catalytic Activity

The iodine - azide reaction is catalysed by free sulphide and diverse metal sulphides, by thiocyanate, thiosulphate and carbon disulphide and by thiols, disulphides and thioketones, among other organic compounds that contain sulphide sulphur. In contrast, the reaction is not catalysed by sulphite, sulphate and organic sulphoxy compounds.[2] Crystalline or coagulated elemental sulphur does not produce the catalysis, but it does catalyse the reaction as a finely dispersed suspension.[3,4]

Hydrogen selenide does not show any catalytic activity,[5] but some selenium compounds, such as *N*-benzoylselenoureas, Ph–CO–NH–CSe–R, where R = piperidino or morpholino, catalyse the reaction, their catalytic activity being lower than that exhibited by the analogous sulphur compounds.[6] However, the catalytic activity of compounds such as $Na_2Se(S_2O_3)_2.3H_2O$ and $Na_2Te(S_2O_3)_2.2H_2O$ seems to be due almost exclusively to the presence of thiosulphate ions.[7]

Certain charcoals and carbon blacks also show catalytic activity, due to the presence of active CO_2 complexes in their composition.[8]

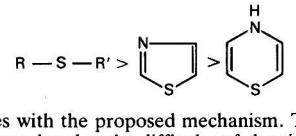
The mechanism of the reaction has been studied by several authors.[9–17] Dahl and Pardue,[16] studying diverse disulphides and mercaptans, established a reaction pathway that explains the behaviour of many catalysts. In the mechanism suggested, the reaction begins with the attack of the $I_2N_3^-$ complex by a disulphide and consists of six reaction steps:

$$I_2N_3^- + RSSR \rightleftharpoons RSI + RSN_3 + I^- \quad (a)$$
$$RSI + N_3^- \rightleftharpoons RSN_3 + I^- \quad (b)$$
$$RSN_3 + N_3^- \rightleftharpoons 3N_2 + RS^- \quad (c)$$
$$RS^- + I_2N_3^- \rightleftharpoons RSI + I^- + N_3^- \quad (d)$$
$$RSI + RS^- \rightleftharpoons RSSR + I^- \quad (e)$$
$$RSN_3 + RS^- \rightleftharpoons RSSR + N_3^- \quad (f)$$

Steps (*a*), (*e*) and (*f*) represent themselves a catalytic cycle, whereas steps (*b*), (*c*) and (*d*) are an inner cycle. The reaction pathway for sulphydryl compounds is the same, but step (*d*) would be the starting point. In this instance, an initial rapid period is observed, due to the high concentration of the RS^- species. The reaction slows down when this species is completely oxidised to disulphide as step (*a*), where the reactive intermediates are regenerated, is the principal rate-determining step.[16] Many other catalysts, such as inorganic sulphides, thioureas and dithiocarbamates exhibit a similar behaviour.[18–20] After some time, the catalysed reaction can stop completely owing to further oxidation of the sulphur to inactive species.

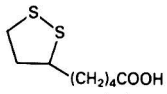
The following order of decreasing catalytic activity has been observed:[21]

$$RSH > RR'C{=}S > R{-}S{-}S{-}R' >$$



which agrees with the proposed mechanism. The differences in activity are related to the difficulty of cleaving the different

bonds to form the RS^- species. Thus, the unexpected high catalytic activity exhibited by lipoic acid:



may be explained by the strain existing on the five-membered ring.[16]

A study performed with different dithio acids of the form $RR'PS_2H$ shows that the catalytic activity decreases when steric hindrance exists on the sulphur atom.[22] The same effect is observed when the active sulphur can form intramolecular hydrogen bonds.[16,23] Thus, for example, cystamine, which differs from cystine only by its lack of a carboxyl group on each extreme of the molecule, exhibits a catalytic activity 2.05 times larger.[16]

On the other hand, electrophilic groups enhance the catalytic activity, which may be attributed to the greater susceptibility of the disulphide bond to nucleophilic attack.[16] Thus, dithiodiglycollic acid, which differs from cystine in its lack of an amino group on each side of the disulphide bond and in the fact that carboxyl groups are on the α-carbons instead of on the β-carbons, shows a catalytic activity 3.2 times larger than cystine. The increase in activity may be explained by the greater difficulty in forming intramolecular hydrogen bonds and by the closer proximity of the electrophilic carboxyl groups to the disulphide bond.[16]

Müller *et al.*[23] established correlations between the structure of several *N*-benzoylthiourea derivatives (RR'N–CS–NH–CO–Ph) and their catalytic activity. Disubstituted compounds are generally ten times as active as monosubstituted compounds, and among the latter, compounds with electron-withdrawing groups are more active than those with electron-donating groups.

Induction Coefficient and Sensitivity

Because of the oxidation side reactions of the catalysts, it is convenient to distinguish between catalytic activity and the induction coefficient. The catalytic activity is a value proportional to the rate constant of the catalysed reaction and to its initial rate, whereas the induction coefficient[24] or reactivity number[25] has been defined as moles of iodine consumed per mole of active sulphur initially present. Therefore, it depends not only on the activity of the catalyst, but also on its resistance to oxidation and on reaction time.

Relative catalytic activities are obtained from the measurements of initial reaction rates, whereas the induction coefficient is calculated from the extent of the catalysed reaction after a certain time period.[26] Not always enough attention has been paid to this distinction, ambiguous expressions such as effectiveness, catalytic effect or reactivity being used.[21,27,28]

The induction coefficient of a substance is a measure of the sensitivity that can be reached in its determination.[24,29] Its value depends on the relative rates of the different competitive reactions involved and, therefore, it changes considerably with the experimental conditions. The induction coefficient usually increases with increasing azide and iodine concentrations[19,30]; however, it can decrease when the concentration of iodine exceeds a certain value, as observed for sulphide,[31,32] and dithiocarbamates.[19,27] An increasing iodide concentration usually causes an increase of the induction coefficient, as observed for cysteine[30] and free sulphide,[31–33] although for metal sulphides[20] and thiocyanate,[34] a diminuition of the coefficient is produced above a certain iodide concentration.

Optimum conditions are not always utilised. Thus, for example, moderate concentrations of azide and iodide, much lower than the optimum, are often used in order to achieve cheaper determinations.[19,20,24]

The induction coefficient usually decreases rapidly above pH 8, owing to the dismutation of iodine. It is inconvenient to work below pH 5 owing to the volatility and toxicity of hydrazoic acid (log K_H = 4.7 at 25 °C, boiling-point 37 °C).[35] In the pH range 5–8 the reaction may be independent of pH, as occurs with ZnS[20] or it may show a more or less marked dependence, as observed for substituted thioureas.[28,36] Some compounds show a maximum and a minimum near pH 5–6 and 7–8, respectively.[16,37] Most procedures recommend a pH in the range 5.5–6.5, where the azide - hydrazoic system shows some buffer capacity.

The induction coefficients may change considerably with the order of addition and even with the rate of mixing of the reagents.[19,38] This could be due to different local iodine concentrations during the process. Thus, for cysteine, a maximum value is obtained when the order of mixing is I^-, N_3^-, HCl, cysteine and I_2, being only half as much if cysteine is added last.[39] Dependences on the dielectric constant, viscosity[27] and total saline content[40] have also been reported.

The induction coefficients of a variety of substances in different experimental conditions have been established.[41,42] Most of the catalysts have values below 600, but in some instances they are much higher. Thus, thioammeline (4,6-diamino-1,3,5-triazine-2-thiol) shows a value of 4400 in a 2% azide solution.[40]

Qualitative Analysis

The identification of diverse organic and inorganic sulphur compounds, making use of the catalysis of the iodine - azide reaction, was suggested by Feigl and Anger.[43,44] The test may be performed either on filter-paper impregnated with the iodine - azide reagent or on a watch-glass, where the formation of nitrogen bubbles is also observed, together with the loss of iodine colour.

The limits of detection are in the range 0.02–0.5 μg for sulphide, thiosulphate and carbon disulphide, being about 2 μg for thiocyanate.[2,35] Extremely low values have been obtained for some organic compounds such as thioacetic acid (0.3 ng), rhodanine (3 ng) and thiourea (5 ng).[44]

Some qualitative uses are the detection of mercaptans in bacterial cultures[45] and sulphur compounds in urine, which is used for the diagnosis of intoxication by dithiocarbamate fungicides[46] and by accelerators of rubber polymerisation.[47] A method has been proposed to differentiate between animal and vegetable fibres (*e.g.*, wool and cotton). After melting with sodium or potassium, only animal fibres give positive results.[48] Finally, an iodine - azide solution is employed to develop sulphur compounds in column adsorption[49] and paper chromatography.[50]

Quantitative Analysis

The reaction has been widely used to determine small amounts of sulphur compounds and has also been applied to the determination of metal ions that form stable complexes with sulphur-containing ligands. The diverse procedures proposed for the determination of non-metallic inorganic and organic compounds are examined in this section, and their detection limits and other characteristics are shown in Table 1.

The analytical applications of the reaction are determined by the oxidation side reactions of the catalysts. In only a few instances, under restricted conditions and during short periods, may it be considered that the concentration of the catalyst is constant and that the reaction proceeds according to definite simple kinetics. For this reason, most of the procedures described have been developed on a purely empirical basis, without establishing the corresponding kinetic equations. In most of these procedures, an excess of iodine is rapidly added to the stirred mixture of azide and catalyst, some control over addition and stirring rates[24] and temperature being necessary. Many analytical techniques based on the measurement of unreacted iodine, evolved nitrogen or heat produced have been used to monitor or establish the advance of the reaction.

Table 1. Determination of non-metallic inorganic and organic compounds

Substance determined	Method	Characteristics of the procedures, samples and references
S^{2-}	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	After 15 min of reaction, determination of 0.03–3 p.p.m. and 3–300 p.p.b. using 0.02 M/0.01 M and 5 mM/2.5 mM iodine - azide solutions, respectively; CV* < 6%. Previous separation as H_2S by sweeping with nitrogen; volatile mercaptans interfere.[51] Similar procedures applied to industrial waste waters, sewage[52] and steel[31] with LOD† 0.5 p.p.b.; air (LOD 1.5 μg)[53,54] and bile[55]
	Titration with arsenite after a fixed time with amperometric end-point detection	Determination of 0.2–200 p.p.m.[56]
	Gasometry	Determination of 1–200 μg. Water and water - organic solvent mixtures[38,57]
	Spectrophotometric determination of iodine in the presence of starch after a fixed time	After 10 min of reaction. Applied to Ti and Zr.[58] Similar procedure for steel[32]
	Turbidimetry of nitrogen and enthalpimetry	Determination of 300–3000 p.p.m. in 100 μl, and 0.1–100 p.p.m. if H_2S is recovered from a nitrogen stream. Applied to the measurement of bacterial activity of *E. coli* on cysteine[59]
	Appearance of fluorescence up to a fixed value	Determination of 0.5–1 p.p.m. in 1 ml. Rhodamine B recommended as indicator[60]
	Direct injection enthalpimetry	After 2 min of reaction, determination of 1.2–32 p.p.m. in 0.5 ml, CV < 3%. Previous separation as H_2S by sweeping with nitrogen; applied to commercial copper with a CV < 5%[33]
	Potentiometric sensor with two platinum microelectrodes	Determination of 8–1000 ng, CV 10–2.7%, LOD 5 ng. For H_2S in a gas stream; considerable amounts of SO_2, HCHO, CS_2 and methylmercaptan can be tolerated[61]
	Coulometric generation of iodine	Determination of 4–40 p.p.b. in 25 ml, CV 2%[62]
	Addition of iodine in the presence of ascorbic acid. Biamperometric end-point detection	Determination of 0.3–1.6 p.p.m. in 50 μl, CV 2.5% for 1.3 p.p.m., LOD 10 ng[63]
	FIA method with biamperometric determination of iodine	LOD 0.2 p.p.m. in 10 μl[64]
Elemental sulphur	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	Determination of 0.1–3.5 p.p.m. in 50 ml, CV < 0.24%. Previous extraction in a miscible organic solvent. Applied to metallic sulphides,[65,66] metals and alloys[67] with CV < 4%, ointments,[4] rubber and polymers with CV < 6%[68–70]
	As above	Determination of 5–80 μg in 1–10 ml of extract, CV < 4.9% in the range 6.6–31.8 μg. Previous extraction in DMF and oxidation to $S_2O_3^{2-}$ with nitrite. Applied to carbonates[71]
$S_2O_3^{2-}$	As above	Determination of 2 μg–0.3 g, CV <2.5%. Thiocyanate is masked with KI[34,72]
	As above, with amperometric end-point detection	Determination of 2–800 p.p.m.[56]
	Effects derived from nitrogen bubble formation (see text)	Determination of 4–40 p.p.m. in 12 ml[73]
	Turbidimetry of nitrogen and enthalpimetry	Determination of 320–3240 p.p.m. in 100 μl (batch method) and 112–1120 p.p.m. (flow method)[59]
	Appearance of fluorescence up to a fixed value	Determination of 0.5–3.5 p.p.m. in 1 ml. Rhodamine B recommended as indicator[60]
	Coulometric generation of iodine	Determination of 20–280 p.p.b. in 25 ml, CV < 2%,[62]
	FIA method with biamperometric determination of iodine	LOD 0.1 p.p.m. in 10 μl[64]
$S_4O_6^{2-}$	Gasometry	LOD 0.01 μg, CV 2–3%.[74] Similar procedure for mixtures with thiosulphate, previous separation by paper chromatography[75]
SCN^-	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	After 30 s of reaction, determination of 2.5 p.p.b.–70 p.p.m., CV 2.5%[76]
	As above, with amperometric end-point detection	Determination of 2–3500 p.p.m.[56]
	Gasometry	—[57,77,78]
	Spectrophotometric determination of iodine at 350 nm after fixed time	After 3 min of reaction, determination of 5–80 p.p.b.[79]
	Appearance of fluorescence up to a fixed value	Determination of 0.2–6 p.p.m. Rhodamine B recommended as indicator[60]
	Coulometric generation of iodine. Biamperometric end-point detection	Determination of 20–120 p.p.b. CV < 3%. In mixtures with thiourea[80]
	Amperometric determination of iodine	Determination of 10–100 ng[81]
	Turbidimetry of nitrogen and enthalpimetry	Determination of 0.7–7 g l^{-1} in 100 μl[59]

Table 1—*continued*

Substance determined	Method	Characteristics of the procedures, samples and references
$(CH_2SCN)_2$ and $PhCH_2SCN$ ..	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	Determination of 2–20 p.p.m. and 4–40 p.p.m. in 1 ml, respectively. Applied to contaminated air[53,54]
3-Butenyl isothiocyanate ..	As above	After 30 s of reaction. Microdetermination in a half rape seed, previous synthesis of the corresponding thiourea with methylamine[82]
CS_2	As above	After 30 min of reaction, determination of 20–120 p.p.m. in 5 ml. Determination of mixtures with ethyl xanthate in sewage[83,84]
	As above	After 30 min of reaction, determination of 0.05–1 μg, CV 5%. Industrial wastes.[85] Similar procedure for air[53,54]
	Fixed signal method. Bleaching time is measured	Bleaching times are in the range 6–100 min. Determination of 0.005–1.5% CS_2 in 1 ml of organic solvent. Mercaptans and H_2S interfere.[86] Similar procedures for 7.5×10^{-4}–4×10^{-3}% in 1 ml of benzene and for 0.1–2.25% in 1 ml of benzene or chloroform[54]
Cysteine	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	After 4 h of reaction, determination of 0.4–2.4 p.p.m. (with 1 g of NaN_3 at pH 6) and 0.1–0.8 p.p.m. (with 2.5 g of NaN_3 at pH 5.2) in 50 ml, CV < 5%. In mixtures with glutathione[29] and ergothioneine.[29,87] In erythrocytes, previous separation by gel chromatography[5]
	As above	After 20–30 s of reaction, determination of 13 p.p.b.–2.4 p.p.m. in 75 ml, CV 4%. Cystine and methionine up to 0.8 and 0.2 mg, respectively, can be tolerated. Applied to milk, beer and wheat.[88] Similar procedure in ref. 30.
	Gasometric measurement after a fixed time	After 10–60 min of reaction, determination of 24–120 p.p.m. in 2 ml, CV 8%[15]
	Spectrophotometric determination of iodine in the presence of starch after a fixed time	LOD 8.6 p.p.b. in 7 ml[89]
	Turbidimetry of nitrogen and enthalpimetry	Determination of 350–3500 p.p.m. in 100 μl[59]
	FIA method with biamperometric determination of iodine	LOD 0.2 p.p.m. in 10 μl[64]
	Coulometric generation of iodine	Determination of 2–16 p.p.b. in 25 ml, CV <2%. In albumin[90]
	Addition of iodine in the presence of ascorbic acid. Potentiometric end-point detection	CV 4.6% for 28 ng of S as cysteine[23]
Glutathione	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	After 5 min of reaction, determination of 2–12 p.p.m. (with 1 g of NaN_3 at pH 6), and 1–5 p.p.m. (with 2.5 g of NaN_3 at pH 5.2) in 50 ml, CV < 5%. In mixtures with ergothioneine,[87] and cysteine and ergothioneine.[29] In erythrocytes, previous separation by gel chromatography.[5] Similar procedure, after 15–20 s of reaction, in 0.5–3 ml of lemon and orange juices[91]
	FIA method with biamperometric determination of iodine	LOD 0.2 p.p.m. in 10 μl[64]
	Coulometric generation of iodine	Determination of 40–240 p.p.m. in 25 ml, CV < 2%[90]
Ergothioneine	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	After 5 min of reaction, determination of 0.02–8 p.p.m. in 50 ml, CV 3%. Cysteine and glutathione are blocked in 5 min with *N*-ethylmaleimide.[29,87] In erythrocytes, previous separation by gel chromatography[5]
2,6-Thio-4-pyrimidine	Addition of an iodine - azide solution at a constant rate	Determination of 10–100 p.p.m. in 10 ml, LOD 2 p.p.m.[92]
2-Mercaptobenzimidazole ..	Spectrophotometric determination of iodine after a fixed time	Determination of 50–200 p.p.b., CV 1.9–5.6%. In zinc electroplating baths. Zn^{2+} is masked with EDTA[93]
6-Mercaptopurine, 2-thiouracil and 2-mercaptopyrimidine ..	FIA method with biamperometric determination of iodine	LOD 0.1 p.p.m. in 10 μl[64]
2-, 6- and 8-mercaptopurine ..	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	Determination of 3–12, 1–12 and 5–80 μg of the three compounds, with CV 2.5, 2.0 and 2.0%, respectively[94]
Thioammeline	As above	Determination of 10–150 p.p.b. in 50 ml, CV 2%. Other mercaptans are blocked with *N*-ethylmaleimide and Cu^{2+} is masked with oxalic acid[95]
Bismuthiol I	As above	Determination of 0.06–1 p.p.m. in 50 ml[96]

Table 1—*continued*

Substance determined	Method	Characteristics of the procedures, samples and references
Methionine	As above	After 30 min of reaction, determination of 7–230 p.p.m. in 50 ml, CV 3%. Cystine up to 20 p.p.m. can be tolerated[97,98]
Ethionine	As above	Determination of 10–700 p.p.m. in 50 ml, CV 1–3%[99]
Sulphathiazole	As above	After 2 h of reaction, determination of 0.3–10 p.p.m. in 50 ml, CV <2%. Drugs[100]
Penicillin G	As above	Determination of 0.2–6 p.p.m. of S in 5 ml, equivalent to 10–350 μg of potassium salt or 15–500 μg of procaine salt, CV 5% for 1 p.p.m. S. The compound is previously hydrolysed in basic medium. Streptomycin and aureomycin do not interfere[101]
Thiamine	As above, with biamperometric end-point detection	After 10 min of reaction, determination of 0.15–2.2 g l^{-1} in 10 ml, CV 2% for 1 g l^{-1}. In drugs, ascorbic acid is titrated separately in absence of azide[37]
Promazine, chloropromazine and promethazine	As above, visual end-point detection in the presence of starch	After 1 h of reaction. Drugs[102]
Cystine	As above	After 30 min of reaction, determination of 1–20 p.p.m. in 50 ml, CV 3%. Insulin, hair, wool and beer.[103] Similar procedure applied to beer; cysteine up to 1.6 p.p.m. can be determined in the same sample; methionine up to 2.7 p.p.m. is tolerated.[88] Similarly, after 2 h of reaction, 0.05–5 p.p.m. (CV 2–3%) can be determined in 100 ml The procedure is applied to insulin, wool and hair[104]
	Gasometry	LOD 1 ng, CV 2.5%[74]
	Spectrophotometric determination of iodine in chloroform extracts at 525 nm after a fixed time. Also gasometry	After 30–60 min, determination of 36–144 p.p.m. in 0.5 ml, confidence limits ±6 p.p.m.; determination of 0.2 ± 0.04 g cystine in samples with 1 g of protein nitrogen (99% confidence level). Applied to human serum protein hydrolysates[14]
	Fixed signal with potentiometric monitoring of iodine	Measured times are in the range 20–200 s; determination of 0.25–2 p.p.m. and 5–25 p.p.m. in 2 ml, CV 1 and 2%, respectively. Alanine, leucine, hystidine and glycine up to 500 p.p.m. and Ca^{2+}, Zn^{2+}, Co^{2+} and Cd^{2+} up to 10^{-3} M do not interfere[105]
	Initial slope method with potentiometric monitoring of iodine	Measurements taken in less than 30 s, determination of 0.25–2 p.p.m. and 2–25 p.p.m. in 1 ml, CV 1 and 2%, respectively[106]
	Turbidimetry of nitrogen and enthalpimetry	Determination of 1.5–15 g l^{-1} in 100 μl[59]
Enerbol	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	CV 1% in drugs[107]
Lipoic acid	As above	Determination of 10–700 μg, CV 1.5%.[108] Similar procedure applied to urine[4]
Thiourea	As above	Determination of 10–30 p.p.m. in 2 ml. Acid copper electroplating baths.[109] Similar procedure for 5–150 μg in citric fruits[4]
	Gasometry	Determination of 2–200 μg in water and water - organic solvent mixtures[38]
	Addition of an iodine - azide solution at a constant rate	Determination of 7–80 p.p.m. in 10 ml, LOD 2 p.p.m.,[92] CV 5%[110]
	Spectrophotometric monitoring of iodine in the presence of starch	Determination of 10–100 p.p.b.[111]
	FIA method with biamperometric determination of iodine	LOD 0.2 p.p.m. in 10 μl[64]
	Coulometric generation of iodine. Biamperometric end-point detection	CV < 3% for 16 p.p.b. In mixtures with thiocyanate[80]
	Turbidimetry of nitrogen and enthalpimetry	Determination of 0.38–3.8 g l^{-1} in 100 μl[59]
	Effects derived from nitrogen bubbles formation (see text)	Determination of 1–10 p.p.m. in 12 ml[73]

Table 1—*continued*

Substance determined	Method	Characteristics of the procedures, samples and references
Thiourea and substituted thioureas	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	After 30 s of reaction, determination of 2–300 μg of S.[112] Mixture of thioureas, previous separation by paper chromatography[50]
	Spectrophotometric determination of iodine in the presence of starch after a fixed time	LOD 3 p.p.b. thiourea and 7 p.p.b. phenylthiourea in 7 ml[89]
	Direct injection enthalpimetry	After 30 s of reaction, determination of 0.05–1 μmol in 100 μl, CV < 3%.[18] Similar procedure for 50–500 μmol in 5 μl, LOD 5 μmol, CV < 2%; applied to the determination of mixtures of thioureas in the presence of mercaptans, isothiocyanates, thiosulphate and sulphide after separation by TLC, CV 6% referred to the sample[36]
Thioureas, substituted thioureas and tetramethylthiuram sulphide	Biamperostatic method	Determination of 2.6–26 nmol thiourea and phenylthiourea, 69–624 nmol benzoylthiourea and 0.6–6.1 nmol tetramethylthiuram sulphide in 5 ml, CV 2.5% for 13 nmol thiourea[25]
2-Thiobarbituric acid	FIA method with biamperometric determination of iodine	LOD 0.1 p.p.m. in 10 μl[64]
2-Thiobarbituric acid and derivatives	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	Determination of 0.02–7 p.p.m. in 100 ml, previous separation by paper chromatography[113]
Rubeanic acid and derivatives	As above	LOD 0.1–0.2 p.p.m. in 50 ml, CV 3–6%[114]
Dithiocarbamates	As above	After 2 min of reaction, determination of 0.1–3.2 p.p.m. sodium diethyldithiocarbamate in 50 ml, CV < 3%; Fe^{3+} and Al^{3+} can be masked with fluoride.[24] Similar procedures for other derivatives,[115,116] and mixtures of *N*-monoalkyl derivatives, previous separation by high-voltage electrophoresis[117]
	Gasometry	Determination of 0.5–100 μg sodium tetramethylenedithiocarbamate in water and water - organic solvent mixtures[38]
	Direct injection enthalpimetry	After 10 s of reaction, determination in the 5–65 μM range in 5 ml, CV 2% at a 30 μM level[19]
Ethyl xanthate	Titration with arsenite after a fixed time. Visual end-point detection in the presence of starch	After 15 s of reaction, determination of 20–55 μg of S. In mixtures with CS_2 in sewage[83]
Merthiolate	Spectrophotometric determination of iodine in the presence of starch after a fixed time	LOD 1 p.p.m. in 7 ml[89]
Azide	Back titration with thiosulphate after total consumption of azide	Determination of 0.1–700 mg of N_3^-.[118] A 15–20 min reaction time in the presence of thiocyanate is recommended[119]

* Coefficient of variation.
† Limit of detection.

Fixed Time Methods

The selection of an optimum reaction time implies a compromise between the current rate and extent of the reaction. When the rate slows down excessively, a longer time of reaction does not yield any valuable gain in sensitivity. The most suitable reaction time can be easily established when a signal - time graph is recorded.

The extent of the reaction is obtained either by titrimetry of the remaining iodine with sodium arsenite[3,56,65,71,98] or hydrazine sulphate,[51] or by a spectrophotometric,[79,93,120,121] enthalpimetric[19,33,36] or gasometric method.[57,77].

A flow injection analysis method (FIA) has been described, in which 10-μl samples are injected into an iodine and azide solution stream. Measurement of the concentration of iodine is carried out biamperometrically.[64]

In enthalpimetric methods, the temperature rise produced by the reaction is proportional to the amount of iodine and azide consumed and, therefore, to the amount of catalyst. These methods are very sensitive, as the iodine - azide reaction is highly exothermic.

Methods based on the measurement of released nitrogen are less sensitive, although together with enthalpimetric methods they offer the advantage over other instrumental methods of not being affected by turbidity or precipitates in the sample.

Fixed Signal Methods

Pardue and Shepherd[105] proposed a fixed signal method for the determination of cystine. A potentiometric concentration cell, sensitive to iodine, is used and the time required for the cell voltage to reach a given value is measured. The concentrations of azide and iodide are large compared to that of iodine and remain essentially constant. Under these conditions, and at cystine concentrations below 2 p.p.m., the rate of the reaction is proportional to the instantaneous concentrations of cystine, C, and iodine:

$$-\frac{d[I_2]_t}{dt} = k_1 C[I_2]_t \quad \ldots \quad (1)$$

where k_1 is a rate constant. When these pseudo-first-order kinetics are obeyed, the time interval Δt required for the voltage interval ΔE to be overcome is inversely proportional to cystine concentration, which may be expressed by

$$C = -\frac{\Delta E}{k k_1} \times \frac{1}{\Delta t} \quad \ldots \quad (2)$$

where k is a temperature-dependent constant from the Nernst equation. However, above 2 p.p.m. of cystine, the reaction order, n, with respect to cystine increases gradually, leading to positive errors. Equation (1) should be rewritten in the form

$$-\frac{d[I_2]_t}{dt} = k_1 C^n [I_2]_t \quad \ldots \quad (3)$$

where $n \geqslant 1$. Equation (2) then becomes

$$C = -\left(\frac{\Delta E}{k k_1}\right)^{1/n} \left(\frac{1}{\Delta t}\right)^{1/n} \quad \ldots \quad (4)$$

which states that C is proportional to the nth root of the reciprocal of the time interval. Under the conditions used and for the range 5–25 p.p.m., when the average value used is $n = 1.24$, the errors are within 2%.

A method has been proposed for the determination of CS_2 based on the measurement of the time needed for iodine to disappear, which may be carried out by visual observation.[86] Rhodamine B has been used as a fluorescent indicator in the determination of sulphide, thiosulphate and thiocyanate. The fluorescence of the indicator, quenched by iodine, appears gradually as the reaction proceeds. The reciprocal of the time required for the fluorescence to reach a certain intensity is proportional to the catalyst concentration.[60]

Initial Slope Methods

Pardue[106] proposed an initial slope method to determine cystine. The initial rate of the reaction is measured by the decrease in iodine concentration, which is monitored potentiometrically. A linear response with a slope proportional to the cystine concentration is obtained. The relationship is given by

$$\frac{\Delta E_t}{dt} = -kk_1 C \quad \ldots \quad (5)$$

The method suffers from the same drawbacks of the corresponding fixed signal method,[105] leading to high values above 2 p.p.m. of cystine.

Open System Methods

Some procedures have been described in which an iodine - azide solution is added at a constant rate to the sample solution. When the catalyst has been completely destroyed, an increase in the iodine concentration is observed. The volume of reagent added is proportional to the initial concentration of the catalyst.[92,110,122,123] The iodine concentration must be kept low and constant in order to achieve reproducible results. Therefore, the addition and stirring rates must be carefully controlled.

A precise and convenient control of the concentration of iodine may be attained by using competitive reactions, coulometric iodine generation or stat methods. Müller *et al.*[23] proposed the addition of iodine in the presence of a given amount of ascorbic acid, which competes with the catalysed reaction, giving rise to a larger consumption of iodine. The time required for iodine to appear in the solution is proportional to the initial concentration of the catalyst. A similar method for microsamples has been described.[63]

Jedrzejewski and Ciesielski[62,80,90] used the anodic generation of iodine. The end-point was detected biamperometrically.

In the biamperostatic method proposed by Pantel,[25] the iodine concentration is measured continuously and a biamperometric signal is used to control the rate of addition from an automatic burette. In this way the iodine concentration is kept constant at a value below 0.2 mM. The determinations are accomplished in a short time.

Other Catalytic Methods

Weisz and Meiners[73] described an unconventional method, where drops of a chloroformic iodine solution are introduced with a capillary into a long and narrow vertical glass tube containing the mixture of azide and catalyst. The nitrogen produced in the catalytic reaction is retained on the falling drops and their downward movement gradually stops. The depth of fall and the time needed for the drops to return to the upper end of the tube are non-linearly related to the concentration of the catalyst.

In the turbidimetric method described by Weisz *et al.*,[59] the measurements are carried out in a 3 + 1 glycerol - water medium, which regularises the formation of nitrogen bubbles. During a given time, and owing to light scattering, the absorbance increases and then decreases, giving rise to a maximum. The height of the maximum and the time required to reach it are both a measure of the concentration of the catalyst, although the relationships are not linear. The ratio of the two parameters gives more precise results than either used separately. The authors employed this method and direct injection enthalpimetry simultaneously to determine several catalysts in batch and flow-through systems (double indication). Enthalpimetric detection provides a very precise indication of the zero time point.

A sensor for determining hydrogen sulphide in a gas stream has been designed.[61] The end of the sensor is a sintered-glass ball, which exudes an iodine - azide solution at a constant flow-rate. Hydrogen sulphide transported by the carrier gas catalyses the reaction, giving rise to a potential difference measured by two platinum electrodes, one inside and the other outside the ball.

Selectivity

The selectivity of most of the procedures is given by the selectivity of the iodine - azide reaction itself. Iodine and iodide oxidants and iodine reductants interfere. However, these interferences may usually be overcome by means of blank determinations in the absence of azide.[37,52] Interferences due to cations that precipitate or complex iodide or the catalyst may be avoided in some instances with masking agents.[24,52,95] The amount of iodine consumed by the oxidation of the catalyst is always negligible.

Procedures allowing the selective determination of different catalysts, found together in the sample, make use of differential kinetic methods of analysis, other selective reactions to block or destroy certain catalysts and previous separation.

Differential Kinetic Methods

Differential kinetic methods can only be applied in some favourable instances, when the behaviour of the catalysts is sufficiently different. A procedure for the simultaneous determination of xanthates and CS_2 in sewage, based on the difference in their induction coefficients, has been described. The extent of the reaction 15 s and 3 min after the introduction of iodine is measured in parallel experiments. The amount of reacted iodine is proportional to the initial concentration of xanthates and to the sum of xanthates and CS_2, respectively.[83]

Mixtures of cysteine and cystine can be resolved by a similar procedure. Cysteine is determined 20–30 s after the beginning of the reaction, whereas the sum of both compounds is obtained after 30 min. The procedure was applied to their determination in milk, beer and wheat.[88]

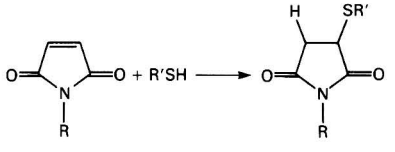
Jedrzejewski and Ciesielski[80] applied their coulometric titration method to the selective determination of mixtures of thiourea and thiocyanate. The method differs from those cited above because the concentration of the generated iodine is controlled instead of the induction time. Thiourea catalyses the reaction at a lower iodine concentration and at a greater rate than thiocyanate.

Finally, the biamperometric sensor of Kiba and Furusawa[61] allows the determination of hydrogen sulphide in the presence of substantial amounts of carbon disulphide and methylmercaptan, owing to their different catalytic activities and the transient nature of the signal. Carbon disulphide and methylmercaptan react more slowly, so that a 100-fold amount with respect to hydrogen sulphide merely broadens the peaks, leaving their heights unaffected.

Masking and Derivatisation

The resolution of mixtures of diverse sulphydryl compounds has been performed making use of their reactions with α,β-unsaturated carbonyl compounds, such as *N*-ethylmaleimide, and with aldehydes.

Mercaptans are added to the *N*-ethylmaleimide double bond, giving thioethers, which have very low catalytic activities:



N-Ethylmaleimide blocks cysteine and glutathione in less than 5 min, whereas no reaction is observed with ergothioneine, even after a few hours. Analytical procedures based on these differences have been described for determining mixtures of the two former compounds with the latter.[87] Similarly, thioammeline can be determined in the presence of other sulphydryl compounds.[95]

Aldehydes also block sulphydryl groups, giving thioacetals:

$$RCHO + 2R'SH \longrightarrow RCH(SR')_2 + H_2O$$

Mixtures of cysteine, glutathione and ergothioneine have been resolved making use of the different rates of reaction with formaldehyde.[29] The percentages of blocked compounds in a 0.3% formaldehyde solution at pH 8–9.5 and after 30 s reaction time are 92, 5 and 27%, respectively, the reproducibility of the blocking percentages being within 2%.

To determine mixtures of cysteine (x) and glutathione (y), a first experiment is carried out after 4 h of reaction time, giving rise to a result, A, proportional to the sum of both substances ($A = C_x + C_y$). In a second experiment the sample reacts with formaldehyde during the 30 s before the introduction of azide. The second result obtained, B, is related to the concentration of the compounds according to

$$B = 0.08C_x + 0.95C_y$$

The concentrations are found by solving the equation system. Similarly, a series of three parallel experiments, using both reagents, formaldehyde and *N*-ethylmaleimide, permits the resolution of mixtures of cysteine, glutathione and ergothioneine.[29]

Sulphite, although it does not catalyse the reaction, reduces iodine, and therefore interferes with the determination of sulphide. This interference may be avoided in a similar way by blocking sulphite with formaldehyde.[121]

The selective determination of isothiocyanates in the presence of other catalysts of the iodine - azide reaction may be performed by treating the sample with an amine. The corresponding thiourea, which is a much more active catalyst, is formed. The micro-determination of 3-butenyl isothiocyanate in a half rape seed has been carried out in this way, after prior extraction of the compound with ethanol. An aliquot of the extract is treated with methylamine to give *N*-methyl-*N'*-(3-butenyl)thiourea, the induction coefficient of which is much greater than the coefficients of the other sulphur-containing compounds present in the seed. A blank determination is also made with an aliquot of the extract not treated with methylamine.[82]

Mixtures of thiocyanate with sulphide or thiosulphate have been resolved making use of the inhibitory action of iodide on the activity of thiocyanate.[34]

Separation Methods

Among the compounds that exert a catalytic action, only H_2S, CS_2 and lower alkyl mercaptans volatilise at room temperature. The determination of sulphide can be performed by sweeping H_2S with a nitrogen stream and absorbing it in dilute NaOH[33,58] or in a zinc(II) or cadmium(II) solution.[51] It is, however, faster and simpler to absorb it directly in the iodine - azide solution.[54]

Interference from thiosulphate and thiocyanate in the identification of the sulphide ion is overcome by precipitation of sulphide with zinc or cadmium carbonate.[2] Similarly, sulphide and thiosulphate are eliminated by precipitation with $HgCl_2$ for the identification of thiocyanate.[35]

Small amounts of elemental sulphur have been determined in metals and alloys,[67] metal sulphides,[65,66] vulcanised rubber,[68,69] sulphur-containing polymers[68,70] and ointments,[4] after extraction with an organic solvent. Elemental sulphur must be finely dispersed to act as a catalyst, which is achieved by using a miscible organic solvent, such as dimethylformamide, and adding an aliquot of the extract into the iodine - azide aqueous solution.[3,65,70]

Thin-layer chromatography has been used to resolve mixtures of substituted thioureas.[36] The separation is performed on silica gel layers with dioxane - benzene - acetic acid (90 + 80 + 1) as the eluent. The spots are located by spraying chromatograms of standard solutions with an iodine - azide solution. The corresponding areas on the chromatogram of the sample solution are scraped off and suspended in an azide solution, and the components are determined by direct injection enthalpimetry.

Substituted thioureas[50] and mixtures of thiosulphate and tetrathionate[75] have been determined after separation by paper chromatography, and high-voltage electrophoresis in a pH 9.2 borax buffer has been used for substituted dithiocarbamates.[117]

The determination of cysteine, glutathione and ergothioneine in haemolysate of erythrocytes has been performed after separation in a Sephadex G-10 column. By elution with a pH 6.8 phosphate buffer two fractions are obtained, the first corresponding to the mixture of cysteine and glutathione,

Table 2. Determination of metal ions

Element	Ligand*	Characteristics of the procedures, samples and references
Hg(I) and Hg(II) . .	Ethylenediamine-dithiocarbamate	Determination of 0.5–8 μg[115]
Co(II)	6-Mercaptopurine	Determination of 1–30 μg[124]
	Pyrrolidinedithio-carbamate	After 10 min of reaction, determination of 0.5–15 μg, CV < 2.5%, previous separation from Ni, Mn, Cu, Fe and Zn by ion exchange[125]
	Diethyldithiocarbamate	After 2 min of reaction, determination of 10–240 p.p.b. in 5–100 ml, CV 5% (9% at 10 p.p.b. level), Fe(III) and Al(III) can be masked with fluoride.[126] Applied to nickel chloride and drugs; Co(II) is previously separated from Ni(II) and Cu(II) by ion exchange[40]
Ni(II)	As above	After 2 min of reaction, determination of 28–280 p.p.b. in 5 ml, CV < 8%. In margarine and drugs: Ni(II) is previously separated from Cu(II) and Co(II) by ion exchange[40]
	As above†	After 10 s of reaction, determination of 60–840 p.p.b. in 5 ml, CV < 3%. In Zamack alloys previous separation by extraction with dimethylglyoxime[19]
	6-Mercaptopurine	Determination of 1–30 μg[124]
Cu(II)	Diethyldithio-carbamate‡	Determination of 6×10^{-6}% Cu in 1 g NaCl[120,127]
	Thioammeline	Determination of 6–250 p.p.b. in 50 ml, CV < 10%. In zinc salts, without separation or pre-concentration of copper[128]
	As above	Determination of 3–24 p.p.b. in 10 ml and 2–240 p.p.b. in 50 ml. In tap and distilled water, commercial azide, acetic and oxalic acids.[129] In drugs, previous separation of Co(II) and Ni(II) by ion exchange[40]
	8-Mercaptopurine	Determination of 0.04–1.6 p.p.m. in 50 ml[130]
	Thiopental	Determination of 20–200 p.p.b. in 50 ml, previous separation of Zn(II) by ion exchange[131]
Zn(II)	As above	Determination of 40–400 p.p.b. in 50 ml, previous separation of Cu(II) by ion exchange[131]
Pd(II)	6-Mercaptopurine	After 10 min of reaction, determination of 0.01–3 p.p.m., CV 4%, but 10% at ng level. Other Pt-group metals can be tolerated in the presence of masking agents[132]
Fe(III)	As above	Determination of 0.1–30 μg[124]
Rh(III)	2-Mercaptopurine	Determination of 0.2–10 p.p.m., CV 3%[133]
Au(III)	As above	Determination of 0.1–0.8 p.p.m., CV 5.1%[134]
	2- and 6-mercapto-purine	LOD 20 p.p.b. in 5 ml[135]
Bi(III)	Bismuthiol	Determination of 0.1–20 p.p.m. in 50 ml, CV 2–10%; Sn(II), Pd(II), Cu(II), Fe(III), Pb(II) and Zn(II) interfere[96]
Ir(IV)	2-Mercaptopurine	Determination of 10–400 p.p.b., CV 8.2%[133]
Pt(IV)	6-Mercaptopurine	Determination of 0.2–5 p.p.m., CV 11%[136]
Ru(VIII) and Os(VIII)	As above	Determination of 0.1–1 p.p.m. Ru(VIII) and 0.02–1 p.p.m. Os(VIII) in 5 ml, CV 6.1 and 6.4%, respectively. Previous separation by volatilisation as RuO_4 and OsO_4[137]
	2- and 6-mercapto-purine	LOD 2 and 5 p.p.b. in 5 ml, respectively[135]

* Except in the instances indicated, the procedure involves the determination of the free ligand by titration of the unconsumed iodine with arsenite after a fixed reaction time.

† Determination of the free ligand by direct injection enthalpimetry.

‡ Extraction of the complex, displacement of the ligand and spectrophotometric determination of the unconsumed iodine after a fixed reaction time.

which in the column are oxidised to disulphides, and the second corresponding to ergothioneine.[5] The selective determination of cysteine and glutathione is carried out as described above,[29] after reduction of the disulphides with H_2Se.

Determination of Metal Ions

Metal complexes with sulphur-containing compounds usually exhibit a lower catalytic activity than do free ligands, owing to the blocking of active sulphur atoms by coordination. Moreover, the catalytic activity of the complexes is often almost cancelled. This inhibition effect may be used in the indirect determination of metal ions, following the extent of the iodine - azide reaction by either of the techniques described above. The characteristics of the proposed procedures are summarised in Table 2.

The inhibition effect may also be used to establish the stoicheiometries and conditional stability constants of the complexes.[126,134,136,138–141]

As corresponds to methods based on an inhibitory effect, calibration graphs show negative slopes and the upper limit of the application range depends on the analytical concentration of the ligand and on the conditional stability constant of the complex. The smaller the constant is, the shorter the calibration linear range will be.[139]

In several instances, side-reactions of complex formation with azide are important and must be considered.[35,126] Although most metal - azide complexes are weak, the use of a large excess of azide can lead to a decrease of the conditional constant.[139]

Metal ion - catalyst - azide equilibria are often attained slowly. Therefore, it may be necessary first to mix these components and to wait some time before starting the catalysed reaction. In the determination of Co^{2+} [126] and Ni^{2+} [19,40] with DDTC, a 10-min waiting time has proved to be adequate.

In addition to the methods based on the inhibitory effect, a direct method for Rh(III), based on the catalytic activity of the Rh(III) - 2-mercaptopurine complex, has been described.[133]

A procedure for determining Cu(II), Pb(II) and Cd(II) on a different basis has also been proposed. The complexes of these metals with DDTC are extracted with chloroform, the excess of ligand is eliminated by washing with NaOH and the extract is added to a methanolic iodine - azide solution. Metal - DDTC complexes are partially decomposed in the presence of an excess of azide, there being free DDTC proportional to the amount of metal. The determination is performed by measuring spectrophotometrically the amount of unreacted iodine 5 min after the beginning of the catalysis. The sensitivity of the method is 50 times better than that of the usual spectrophotometric method, where a direct measurement of the Cu(II) - DDTC absorbance is carried out.[120,127]

The selectivity of the methods for determining metal ions depends on the selectivity of the sulphur compound as a ligand. When this is not enough, interferences may be avoided with masking agents[132] or by previous separation by volatilisation,[137] extraction[19,120] or ion-exchange chromatography.[40,131]

Other Applications

The determination of azide has been performed by titration of the unconsumed iodine with thiosulphate after the total consumption of azide, in the presence of an excess of some stable catalysts.[118] Thiocyanate has been recommended for this purpose.[119]

The iodine - azide indicator reaction has been proposed for end-point detection in titrations with sulphide in acidic media. The first drop of excess titrant causes the evolution of H_2S, which is transferred by a nitrogen stream into a vessel containing the iodine - azide reagent.[142]

The reaction has also been employed for the semi-quantitative measurement of the properties of diverse materials. Thus, the induction coefficients of cyclic tetrasulphides of primary amines have been used to establish cross-linking activity in elastomer vulcanisation. An increase of the molecular weight of the substituents, related to cross-linking activity, leads to higher induction coefficients.[143]

The photographic activity of some sulphur compounds, which are used as additives in photography, is related to their catalytic activity.[23,144] However, methods based on the measurement of the extent of the reaction after a relatively large time period are not suitable as the oxidation side reactions of the catalysts lead to wrong conclusions. Müller *et al.*[23] proposed the use of ascorbic acid to avoid the influence of these side-reactions.

A corrosivity test has been described, based on the observation of nitrogen evolution from surfaces impregnated with the iodine - azide reagent.[145]

Finally, the reaction is useful for studying the stability of sulphur compound solutions of analytical interest in diverse media.[24,146,147]

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Use of Solid Boric Acid as an Ammonia Absorbent in the Determination of Nitrogen

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An inexpensive trap - de-mister assembly utilising solid crystalline boric acid as an ammonia absorbent was developed to replace the specialised trap - condenser - impinger apparatus normally used in Kjeldahl-type distillations. The boric acid is subsequently dissolved in water and a final determination of the ammonium ion is made either by acidimetric titration, a conductance measurement or spectrophotometric measurement after the addition of Nessler's reagent. The apparatus fits into the neck of a calibrated flask during the distillation and is itself constructed from disposable plastic pipette tips, glass-wool and rubber tubing. Both the high surface area and the tortuosity of the paths through the randomly packed bed of crystals makes solid boric acid an efficient ammonia absorbent. The system is useful for small-scale separations only because, when the volume of solution from which the ammonia is distilled exceeds 25 ml, the acid crystals become saturated with water and begin to dissolve before all of the ammonia is trapped.

Keywords: *Solid-state ammonia trap; boric acid; Kjeldahl distillation; nitrogen determination*

Taras's review of nitrogen determination procedures emphasises the importance of what will hereafter be referred to as the "Kjeldahl approach."[1] The two main steps in the Kjeldahl approach are firstly, a chemical conversion of the nitrogen in the sample to ammonia or ammonium ion and secondly, the distillation of the ammonia into an acid liquid trapping agent. The ammonia in the trapping agent is then determined by any of a number of suitable finish techniques. Whereas the original Kjeldahl technique was developed for "organic nitrogen" determinations, the same basic approach can be used for determining practically all of the chemical forms of nitrogen if the distillation step is preceded by a suitable chemical pre-treatment of the sample.

The bottleneck in many of these analytical procedures is the distillation - separation process, not the initial chemical conversion step. Traditionally, this distillation step is performed with specialised all-glass stills that have a water-cooled condenser, the free end of which is immersed in the trapping solution (*e.g.*, the Pregl - Parnas - Wagner apparatus). These distillation systems typically possess high internal surface areas and considerable dead volumes. Consequently, a relatively large volume of water must be boiled over to flush the ammonia released from the sample digest quantitatively through to the trapping agent.

This paper discusses a simple and inexpensive solid-state ammonia trap, which significantly reduces the time necessary for these separations in many situations. An outline of some practical applications is included in order to illustrate the utility of the system.

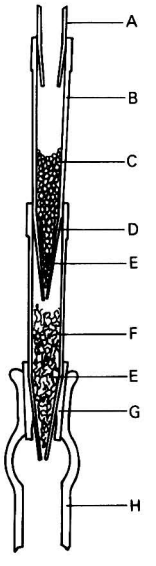

Fig. 1. Trap - de-mister assembly. A, Chimney; B, pipette tip used for body of trap; C, boric acid crystals; D, retainer; E, glass-wool; F, tip used for de-mister; G, gum - rubber gasket; H, neck of distillation flask

Experimental

Apparatus

Fig. 1 shows an ammonia trap assembly inserted into the neck of the distillation flask. It consists of a standard 1-ml disposable plastic micropipette tip, filled approximately one third with boric acid crystals (about 0.5 g). Several batches of boric acid from different manufacturers were used with essentially equivalent results. Crystal sizes were such that more than 95% of the boric acid was retained between sieves passing 850 and 150 μm particles (Tyler 20–100-mesh screens). A plug of glass-wool supports the crystals and a plastic "chimney" prevents steam from pushing the plug of moist crystals out of the top of the trap. The chimney is made by cutting both the top and bottom off another pipette tip. A plastic retainer cut from yet another pipette tip is forced into the trap body over the glass-wool before the boric acid is added. This prevents the glass-wool from being washed out along with the boric acid crystals after the distillation step has been completed.

The ammonia trap is inserted into another pipette tip loosely filled with glass-wool that serves as a "de-mister" to intercept the particles of spray released during the boiling of the sample digestate. The de-mister serves the same purpose as the trap usually incorporated in commercial Kjeldahl systems, but it provides the necessary surface area with far less dead volume and solution "hang up." A short length of gum-rubber tubing is forced over the bottom of the de-mister to serve as a gas-tight gasket between it and the neck of the flask.

Both the de-mister and the trap assembly can be re-used an indefinite number of times. After the boric acid used in a prior

determination has been removed, the system is prepared for the next by connecting it to a sink-aspirator vacuum source and then pulling water, acetone and finally air through it.

Distillation Procedure

Standard 25- or 50-ml calibrated flasks and a magnetic stirrer - hot-plate were used for both the sample preparation and distillation steps. To perform an ammonia distillation, the contents of the digestion flask are first cooled, if necessary, and then diluted to approximately 5 ml with water. If a magnetic stir-bar was not used during the sample preparation step, one is now added to the flask. Alternatively, a few boiling chips can be added. This is necessary because "bumping" can cause expulsive loss of the trap - de-mister assembly and/or its contents. Enough strong base to provide an excess of at least 2–3 mmol of free hydroxyl ion is then added and the filter - de-mister assembly is immediately inserted into the neck of the flask. The flask is placed on to a pre-heated stirrer - hot-plate and its contents are rapidly brought to boiling-point. The trap is removed after an approximately 1.5 min distillation time.

The chimney is pulled out of the trap and any boric acid crystals adhering to it are rinsed into a small beaker containing hot water. The contents of the trap are then back-washed into the same beaker using a plastic wash-bottle with a spout modified to fit snugly over the bottom tip of the trap.

Finish Techniques

For sample digestates containing relatively large amounts of ammonium ion, a titration of the dissolved boric solution proved satisfactory. Standardised hydrochloric acid was added with a 2-ml micrometer burette (Cole Parmer) until the methyl orange visual end-point (*ca.* pH 4) was reached.

For low concentrations, a spectrophotometric finish technique was investigated. This involves the neutralisation of the boric acid with a slight excess of strong base (about 1.1 equivalents of NaOH per mole of boric acid in the trap) followed by the addition of Nessler's reagent and dilution to a suitable volume. The absorbance of the fully developed yellow chromogen was then determined in a spectrophotometer at 425 nm in a 1-cm path-length cuvette.

An alternative and usually superior finish technique consists of the measurement of the conductivity of the boric acid solution. Boric acid is an extremely weak acid ($pK_a = 10.1$) and, consequently, its aqueous solutions are very poor electrical conductors. On the other hand, ammonium borate is a typical ionic salt and gives rise to a relatively large electrical response. For this work, the detection module of a Dionex Model 2000i ion chromatograph was re-plumbed so that the columns were by-passed and the solution could be sucked directly through the conductivity cell with a plastic syringe. After the cell is filled with the solution the measurement is made under "zero-flow" conditions.

Results and Discussion

The initial testing of the solid-trapping agent concept was performed by simply drawing headspace fumes from a reagent "ammonia" bottle through one of the traps with a syringe and then blowing the contents of the syringe past the investigator's nose. The fact that no ammoniacal odour could be detected indicated that boric acid is an effective ammonia absorber.

Table 1 outlines a study performed to determine the effect on ammonia recoveries of using varying distillation times. Aliquots of a standard ammonium sulphate solution were used so that no prior sample preparation step was needed. The amount of ammonium ion taken was chosen to be relatively large (0.1392 mmol) so that the precise titrimetric finish could be employed. The "primary standard" on which the recovery values in both this and the following tables are based is the sodium carbonate used to standardise the hydrochloric acid titrant.

Table 1. Ammonia recovery as a function of distillation time. In all instances the sample consisted of 0.100 ml of 1.392 M ammonium sulphate solution and a total solution volume of approximately 5 ml containing 10 mequiv. of free hydroxyl ion was boiled

Time/s*	Mass of distillate/g	Recovery, %
20	0.35	85.0
30	0.70	99.85
45	0.82	100.01
90	1.40	99.36
150	2.03	100.20
240	3.30	99.71

* The interval from when the condensate first appeared in the de-mister to when the filter was removed.

In this series of experiments the total solution volume in the distillation flask prior to placing it on to the hot-plate was approximately 5 ml. The flask was weighed both before and after the distillation to determine the amount of solution boiled away and passing through the de-mister - trap assembly. The total time required for each distillation was approximately 30 s greater than the figure listed in the first column because that interval was required to bring the solutions to the boil.

The data indicate that, when solution volumes are relatively small, essentially complete ammonia recovery is obtained within 30 s of the onset of rapid boiling. In this instance, this corresponds to 10–15% of the solution in the flask. As might be expected, a series of further experiments performed with different volumes and compositions of the solutions in the distillation flask revealed that the distillation times required for quantitative recoveries were a function of both the volume of the solution being boiled and of its total salt concentration.

As a rule of thumb, quantitative ammonia recovery is achieved by the time that 15% of the aqueous phase has been boiled away. Higher total salt contents raise the boiling-point of the solution, which, in turn, enhances the volatilisation of the ammonia relative to that of the water.

The upper limit to the solution volume from which the ammonia can be effectively trapped is determined by the solubility of the boric acid in the water absorbed/condensed in the trap during the distillation process. After complete water saturation of the boric acid occurs, a portion of the resulting boric acid solution formed subsequently might either drip back into the de-mister or be carried out of the top of the chimney as a spray. This effectively limits the amount of steam that can be passed through the trap to no more than that generated by boiling away approximately 3–4 ml of the solution in the flask. With high salt concentrations (the equivalent of approximately 4 M NaOH) present, ammonia can be quantitatively recovered from a maximum digestate volume of approximately 25 ml. However, to provide a good margin of safety in this respect, solution volumes of no more than 15 ml are recommended.

Tables 2 and 3 give the results of some applications of these traps to nitrogen determinations in a number of different types of compounds. In the experiments described in Table 2, enough of the compound was weighed into a 25-ml calibrated flask to provide 0.3–0.5 mmol of "amino - amido" nitrogen. A 0.5-ml volume of 9 M sulphuric acid was added and the sample was then digested for 5 min on a pre-heated hot-plate with a surface temperature of approximately 400 °C. Under these conditions, the water immediately boils off and the sample digests in quietly refluxing concentrated sulphuric acid at a temperature of approximately 300 °C. No digestion catalyst was used or needed with these readily hydrolysed compounds.

Table 2. Recoveries of nitrogen from various compounds. Samples were digested with 0.25 ml of sulphuric acid for 5 min

Compound	Recovery, %
Iron(II) ammonium sulphate	100.85
Ammonium sulphate	99.97
Thiourea	98.87
Sulphamic acid	99.97

Table 3. Recoveries of nitrate-nitrogen from various compounds. Samples containing approximately 0.4 mequiv. of nitrogen were reduced with aluminium powder in strong base prior to the distillation step. The relative standard deviation of the nitrate determinations performed at this concentration level was approximately 1%

Compound	Recovery, %
Nitric acid	99.89
Cadmium nitrate	99.85
Calcium nitrate	99.67
Sodium nitrate	100.10

The distillation - separation procedure outlined above was then applied.

The excellent nitrogen recoveries obtained for the hydrolysed sulphamic acid samples were of special interest to this laboratory because the original focus of this project was to develop a rapid procedure to determine sulphamate that did not rely on the often troublesome titrimetric determination of nitrite. Indeed, in many instances, the accuracy and precision of sulphamic acid determinations performed with the "Kjeldahl" approach proved to be much better than could be routinely obtained by a more conventional redox titrimetric method involving nitrite.[2] However, as a general-purpose analytical method for sulphamate at this facility, the technique proved to have a major weakness. This is that any concomitant nitrate severely interferes during the hydrolysis step. Nitrate (or one of its decomposition products in the hot sulphuric acid) apparently oxidises some or all of the sulphamate's "amido nitrogen" to elemental nitrogen.

Table 3 shows the results of nitrate determinations performed using the same final separation and finish steps after prior reduction of the sample with metallic aluminium.[3] The procedure involved placing a suitable amount of sample (containing 0.3–0.5 mmol of nitrogen) into a 50-ml flask and then dissolving it in about 2–3 ml of water. Approximately 150 mg of powdered aluminium followed by 2 ml of 10 M NaOH were added and the ammonia trap - de-mister assembly was immediately seated into the neck of the flask. After the initial vigorous reaction had subsided, the flask was placed on the hot-plate and the ammonia remaining in the flask distilled as described above. Recoveries were again excellent.

The spectrophotometric finish approach can be recommended only for those situations in which its excellent analytical sensitivity is actually required by the problem at hand. Nessler's reagent is notoriously difficult to use in high-salt sample solutions and in this work also tended to give erratic results unless considerable care was taken to reproduce exactly all the relevant conditions in preparing the solutions for the spectrophotometer. The absolute and relative amounts of boric acid and base used, solution temperatures, reagent addition technique, reagent volume, etc., all need to be reproduced carefully for good over-all analytical precision. Preliminary experiments also revealed that the "phenate" procedure often recommended for water analyses was even less satisfactory.[4] Both boric acid and borates apparently inhibit the development of the indophenol chromogen.

The most convenient finish technique proved to be the conductance measurement. It was developed to serve as a substitute for the more troublesome Nesslerisation technique for low-nitrogen samples. The mean conductance of a series of six blanks run through the distillation procedure was 7.15 μS and the standard deviation was 0.53 μS. In each instance 0.50 ± 0.02 g of boric acid was used in the traps and the final solution volume was 50 ml. Essentially identical conductance values (a mean of 7.21 μS with a standard deviation of 0.48 μS) were obtained with 1% boric acid - water solutions prepared directly without the intermediate distillation and transfer steps. This indicates both that the de-mister does a very effective job of preventing spray carry-over and that, with reasonable care, avoidance of contamination of the boric acid with miscellaneous salts throughout the handling process is possible.

The analytical response generated by ammonia added to these solutions is approximately 5.6 μS per p.p.m. of ammonia and the response is linear to at least the 1000 μS maximum conductivity range of the detector used for this work. This translates to an analytical range from approximately 0.25 p.p.m. (the detection limit based on the 3σ criterion) to about 180 p.p.m., a range encompassing trace to macro levels of nitrogen in the original sample.

Conclusions

The limited solution volume from which ammonia can be quantitatively recovered probably constitutes the most serious limitation of the ammonia traps. This factor effectively limits their application to relatively small-scale work only. However, for many applications the fact that their use permits good analytical results to be obtained quickly with otherwise non-specialised glassware and equipment makes them an attractive alternative to classical Kjeldahl still set-ups.

The most serious limitation of the conductance finish technique is its lack of specificity. Any ionic species present either in the original boric acid or inadvertantly picked up during transfer steps can give serious blank problems. However, with reasonable care, it is not too difficult to avoid contamination.

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Automatic Nitrogen-15 Analyser for Use in Biological Research

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An automatic nitrogen-15 analyser capable of analysing 12–20 samples per hour has been developed. The generation of pure nitrogen gas from the sample is carried out automatically, the nitrogen isotope ratio is determined by emission spectrometry and the results are calculated as atom-% nitrogen-15. The total nitrogen content of the sample is also determined. Sample masses can vary between 0.5 and 100 mg and only 2–10 μg amounts of N are necessary for accurate determinations.

Keywords: *Automatic nitrogen analysis; emission spectroscopy; nitrogen-15 isotope ratio; automated data handling*

Nitrogen is one of the most important and familiar elements in the biological sciences, as it is one of the main constituents of amino acids, peptides, proteins and nucleic acids. It is conspicuous, however, that little work has been carried out on the kinetics of the assimilation of nitrogenous compounds into cells, their quantitative partitioning between pools and their flow to different metabolites. An important reason for this apparent lack of interest is that all the radioactive isotopes of nitrogen have short half-lives; the longest is only 10 min, for nitrogen-13. The use of radioactive isotopes is therefore both difficult and expensive, although nitrogen-13 has been utilised to study ammonia assimilation pathways by different bacteria.[1–5]

The stable isotope nitrogen-15 has been used for studying the kinetics of ammonia assimilation by *Derxia gummosa*[6] and for the dynamics of nitrogen metabolism in ruminants.[7] Nitrogen-15 has also been used extensively in plant and soil studies.[8,9]

In most of these studies, isotope-ratio mass spectrometers have been used to measure nitrogen-15; this is a relatively expensive and slow procedure, although a high accuracy can be obtained. Broida and Chapman[10] introduced a photoelectric method to measure nitrogen-15 abundance and it became evident from this that emission spectrometry could be very useful for the isotopic analysis of microgram amounts of nitrogen. It has several advantages over mass spectrometry in that an emission spectrometer is less expensive than a mass spectrometer, less demanding of the operator and the minimum amount of nitrogen required for determination is much smaller.[11,12] Since 1958, emission spectrometry has been developed and applied to agricultural studies and many other research areas.[12–16] However, the methods for the preparation of samples to isolate nitrogen for isotopic analysis have so far been time consuming, and have proved to be a limiting factor in extensive surveys such as nutritional studies.[17]

Goulden and Salter[16] devised an automatic emission spectrometer capable of analysing 60 samples per hour. However, biological samples had to be subjected to Kjeldahl digestion and ammonium chloride ultimately recovered, which was then injected into a reactor tube where nitrogen was generated and passed into the discharge tube of the emission spectrometer. This is a tedious process that has to be carried out manually, and on average one operator can only process and measure 70 samples per week.[12] This paper describes a fully automated system that measures total nitrogen and nitrogen-15 in biological materials without prior sample preparation.

Experimental

The system consists of two major components: an automatic nitrogen analyser for determining total nitrogen and an emission spectrometer for measuring nitrogen-15.

Automatic Nitrogen Analyser

The automatic nitrogen analyser (Model NA1500, Carlo Erba Strumentazione, Milan, Italy) was used with a minor modification to the outlet tube for coupling to the emission spectrometer. A schematic diagram is shown in Fig. 1. This instrument is designed for micro and macro determinations of total nitrogen present in a wide range of organic and inorganic substances in both solid and liquid form. The analyser can operate completely automatically and is capable of performing 50 analyses sequentially, using the automatic sampler. It has been used with great success over a wide range of nitrogen concentrations from 10 μg to 2 mg. The instrument operates on the principle of the Dumas combustion procedure. The sample is placed in a tin container and introduced into the automatic sampler, where it is purged with helium for 20 s before it drops into the combustion column, which is maintained at 1000 °C. The container melts and the heat of the reaction of the tin with a small amount of pure oxygen, introduced simultaneously, primes the flash combustion of the sample at *ca.* 1700 °C. Under these conditions, even thermally resistant substances are completely oxidised. The combustion products are carried by a constant flow of helium through chromium oxide granules maintained at 1000 °C. The oxi-

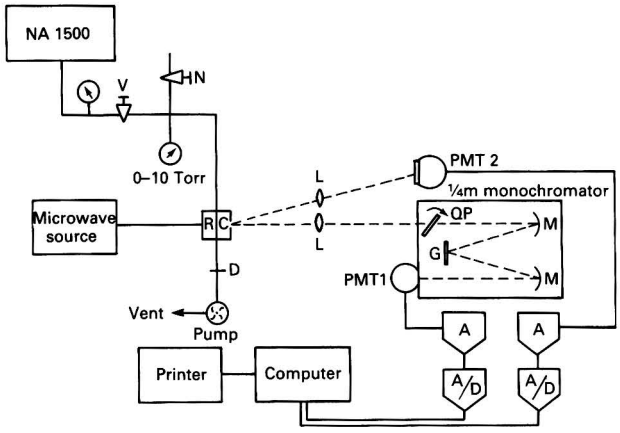

Fig. 1. Schematic diagram of the automatic nitrogen-15 analyser

dation is completed in a 5-cm layer of silver-coated cobalt oxide at the bottom of the combustion column. This also retains interfering substances produced during the combustion of halogenated compounds.

The combustion products, a mixture of CO_2, various oxides of nitrogen and H_2O, pass through a reduction reactor filled with reduced copper maintained at 650 °C. At this temperature the nitrogen oxides are reduced to elemental nitrogen, which together with the CO_2 and H_2O pass through two absorbent filters, the first containing $Mg(ClO_4)_2$ to absorb water, and the second containing NaOH absorbed on asbestos particles to retain CO_2. The elemental nitrogen enters the chromatographic column and, together with the carrier gas, flows through the thermal conductivity detector, which generates an electrical signal proportional to the concentration of nitrogen present. The signal is amplified, the area integrated and the result printed out by the data processor (DP110, Carlo Erba). By calibrating the instrument with a standard, it is possible to calculate the percentage of nitrogen in the samples.

Emission Spectrometer

The analysis of nitrogen-15 by emission spectrometry depends on the property of nitrogen, at reduced pressure (2–10 Torr), to emit light of characteristic wavelengths when energised by radio- or microwaves. The presence of an extra neutron in the ^{15}N nucleus causes shifts resulting in a readily measurable displacement of the bandhead that usually appears in the ultraviolet region from 297.7 nm for the $^{14}N^{14}N$ molecule to 298.3 nm for $^{14}N^{15}N$ and 298.8 nm for $^{15}N^{15}N$. These bands can be readily separated by a small monochromator with *ca.* 1 Å resolution. The intensities of the bands are proportional to their nitrogen content so that in an equilibrium mixture of the three molecular forms of nitrogen, the proportion of ^{15}N can be calculated from the ratio of the bands of mass 28 and 29 in a manner analogous to that used in mass spectrometry.[12]

The spectrometer features a microwave source for the excitation of the nitrogen gas in a stream of He carrier gas, a monochromator that scans rapidly and repeatedly over the relevant spectral region and a computer for the rapid acquisition of spectral information and data processing.

Scanning Monochromator

A Jarrell-Ash 0.25 m Ebert monochromator (Jarrell-Ash Division, Fischer Scientific, Waltham, MA, USA), with a 2360 lines mm^{-1} grating blazed for 300 nm and a dispersion of 1.65 nm mm^{-1} was used. Wavelength scans obtained with the monochromator using slits of 25, 50 and 150 μm, respectively, with a helium and natural nitrogen mixture excited by a microwave discharge at 20 W, showed that the 50 μm slits were the best set to use, as with these there was only a marginal loss in the definition of the spectrum compared with the 25 μm slits. With the 150 μm slits the resolution was definitely impaired.

The rapid scanning over the spectral region was accomplished with a rotating quartz plate QP (1.9 mm thick) behind the entrance slit (Fig. 1). The refraction of the light beam (at approximately 300 mm) is such that a 40° rotation of the plate shifts the image at the exit slit by 0.6 nm, the difference in wavelength between the two peaks to be measured. A synchronous motor is used for driving the refractor plate, in order to ensure the constant speed of rotation that is necessary as the computer measures intensity at regular intervals (700 μs between measurements), so that the two peaks are separated on a time basis rather than on a wavelength basis. Constant rotational speed also eliminates the effect of a 50 Hz ripple on the emission from the microwave source and on the output of the amplifier. The speed of rotation is 1 rev s^{-1}, resulting in a time lapse of 0.11 s between the measurement of the two peaks.

The dispersed signal by photomultiplier tube 1 (PMT1) is constantly related to a signal measured by photomultiplier tube 2 (PMT2) as shown in Fig. 1. The latter measures the intensity of the 337.1 nm bandhead of nitrogen belonging to the same band system, through a narrow-band interference filter that excludes any extraneous light. This serves as an efficient reference for the analytical channel to eliminate the effect of the evolutionary nature of the nitrogen content of the gas mixture given off by the source.

Excitation Source

A Microtron 200 Mark III (Electro-Medical Suppliers, Greenham, UK) microwave generator (2450 MHz) was used for the excitation of the gas mixture. The instrument is equipped with a power meter, a reflected power meter and a magnetron protection cut-out device that becomes operative when the reflected power exceeds 75 W. A 214L type resonant cavity accommodating discharge tubes of up to 13 mm diameter (RC) was used as the termination on the coaxial cable (Fig. 1). The source can supply power to a maximum of 200 W, but the minimum of 20 W is normally used.

Pumping System

The Carlo Erba nitrogen analyser supplies the nitrogen - helium gas mixture at a flow-rate of approximately 80 ml min^{-1}, at atmospheric pressure. Inside the discharge tube the pressure must be *ca.* 5 Torr and the reduction in pressure is accomplished using a vacuum pump (Fig. 1). The pumping speed of the rotatory vacuum pump (Alcatel 2004 A, CIT Alcatel, Paris, France) is reduced by a fixed diaphragm, D (*ca.* 1 mm diameter), in the line. The discharge cavity is located between this diaphragm and the needle valve, V, which is adjusted so that the combined effect of the pump, D and V is such that the pressure on the supply side is near to atmospheric pressure, in order not to interfere with the operation of the nitrogen analyser. A Pirani gauge type pressure meter (Alcatel API 101 T, CIT Alcatel, Paris, France) is available for measuring the pressure in the discharge section, whereas a diaphragm type meter measures the pressure on the inlet side. A second needle valve, N, is available for bleeding air into the system. As this is a constant source of nitrogen for the discharge, rather than one changing with time as supplied by the nitrogen analyser, it can be conveniently used for preliminary measurements and for checking the operation of the system.

Electronics

A single stabilised high-voltage supply (Tennelec C 952, with <3 mV regulation and less than 0.001% drift per hour, 0–10 mA current) is used for the Hamamatsu 1P28 photomultiplier on the monochromator and the Hamamatsu R166 solar blind photomultiplier tube on the reference channel. The light flux is sufficiently high so that the photomultiplier tubes can be used with a high voltage supply of approximately 360 V.

Each photomultiplier signal is amplified and converted by a 12-bit analog to digital converter. The parallel digital information is loaded into a Zenith microcomputer (Zenith Data Systems, Illinois, USA) via two parallel interface ports. An optical switch on the rotating quartz plate drive supplies a reference pulse to the computer that initiates a measurement cycle. In this way the measurements are synchronised with the quartz plate position and therefore with wavelength. The computer triggers the A/D converters for each conversion.

Measurement by Computer

Analysis of a sample begins when the NA1500 cycle is started at time t_0. After the time interval necessary for the gases to pass through the instrument, the first detectable nitrogen appears at the microwave excitation source at time t_1 and is present until time t_2. Programmed measurements begin at t_a and end at t_b [Fig. 2(*a*)]. Ideally, this interval should include the region of maximum intensity.

The programme runs through 25 cycles of measurements in the interval t_a–t_b. Each cycle duration is 1 s (the period of revolution of the rotating quartz plate), but measurements are made only during a fraction of this 1 s period, *viz.*, over 80° or an 80/360 s period. During this period the spectral region of interest, *i.e.*, 297.4–298.6 nm, is scanned.

Only three values of intensity are required from the spectrum, *viz.*, the intensity at the strong $^{14}N^{14}N$ peak at 297.7 nm, the intensity at the weak $^{14}N^{15}N$ peak at 298.3 nm and a background value. The first value is easily selected by the computer from the 320 elements stored in the memory of channel 2 by looking for the maximum of the $^{14}N^{14}N$ peak [I_p, Fig. 2(*b*)]. Next the intensity at the $^{14}N^{15}N$ peak is located 56 measurements after the first peak (I_p +56) and this separation remains fixed. Similarly, the 93rd measurement after the first peak (I_p +93) represents a reliable position (wavelength) at which to measure the background intensity [Fig. 2(*b*)]. These three values from channel 2 are normalised with respect to channel 1, the reference channel, by dividing by the corresponding values of the averaged set of 25 reference intensities.

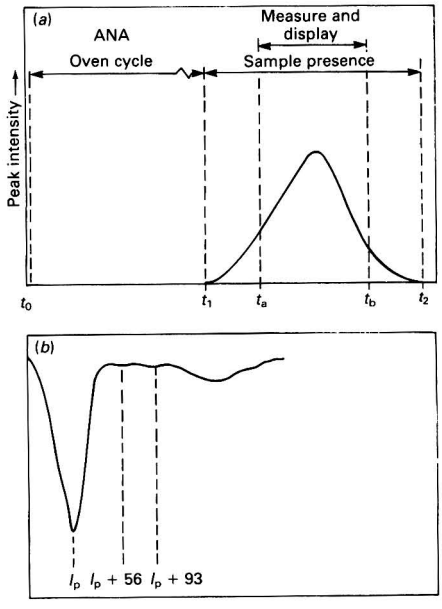

Fig. 2. (*a*) Sequence of events during an analytical cycle; and (*b*) N spectrum showing the positions at which measurements are made

Results and Discussion

Evaluation of Data

Although the background on the long wavelength side of the $^{14}N^{15}N$ peak appears flat and reproducible, it does not represent the real background at the wavelength of this peak, as using this value for calculating the $^{14}N^{15}N$ to $^{14}N^{14}N$ peak ratio yields a value of *ca.* 1 : 50 instead of the value of 1 : 137 for natural (0.365 atom-%) ^{15}N abundance. This is due to the fact that the emission peak of the $^{14}N_2$ molecule at 297.7 nm produces a "skirt" of spectral emission towards the long wavelength side that interferes at 298.3 nm, the wavelength of the $^{14}N^{15}N$ molecule maximum. This is a real feature of the spectrum and not due to inadequate resolution by the monochromator. A sample of known ^{15}N abundance is therefore used to establish the background value. For this purpose a sample at natural abundance has proved to be adequate.

Measurements are made as follows: the intensity of radiation is measured at the $^{14}N^{14}N$ peak, the $^{14}N^{15}N$ peak and at a minimum in the spectrum on the long wavelength side. These values are called P_1, P_2 and B, respectively. The concentration of ^{15}N, C, is given by

$$C = \frac{100}{2R+1} \text{ (in atom-\%)} \quad \ldots \quad (1)$$

where

$$R = \frac{P_1 - A}{P_2 - A} \quad \ldots \quad (2)$$

For a sample of natural abundance, R = 137, and the value of the true background A is given by

$$A = \frac{RP_2 - P_1}{R-1} \quad \ldots \quad (3)$$

Comparing this value with B, the background measured away from the peak, it was found that the ratio $k = A/B$ was both positive and constant, regardless of the signal sizes. The signal size was varied in a 1 : 8 range by varying the nitrogen concentration and also by varying the high voltage on the photomultiplier tube, but no correlation of this ratio with the signal size was found.

After establishing the value of k accurately by running three or more samples of natural abundance, samples of unknown ^{15}N content can be run and the concentration calculated from equation (1) by substituting kB for A, the true background, in equation (2), so that R can be defined in terms of the background measured away from the peak (B), and the concentration of ^{15}N (C) calculated without needing to know the true absorbance. Hence,

$$C = \frac{100}{2R + 1}$$

where

$$R = \frac{P_1 - kB}{P_2 - kB}$$

Following this procedure and running a number of natural abundance samples as unknowns, a standard deviation of better than 0.02 atom-% of ^{15}N is usually found.

Repeated analyses were carried out on defined inorganic and organic substances to test the accuracy and reproducibility of the results obtained with the nitrogen analyser (ANA). These are shown in Table 1. Excellent reproducibility was obtained with all three substances, with coefficients of variation between 0.3 and 0.85%. The agreement between the known nitrogen content of the compounds and the values obtained was very good. The difference was 0.05 for $(NH_4)_2SO_4$, whereas it was 0.08 for haemin, an organic compound from which it is difficult to release nitrogen. The difference for albumin, an organic macromolecule, was only 0.01.

Table 2 shows a comparison of nitrogen analyses of biological samples performed using the Kjeldahl method and the ANA, respectively. Coefficients of variation varied between 1.5 and 2.7%, which is good, considering the heterogeneity of the samples and, with the maize cell walls, the extremely low nitrogen content. It is important to note that the accuracy with which the sample is weighed into the tin cup will influence the performance of the ANA. The use of large samples would therefore increase the accuracy of the method although the size of the tin cup dictates the sample size to a great extent, especially when using biological samples of low density. The sample masses shown in Table 2 were the maximum that could be fitted into the sample cups, except for the duodenal contents and sheep faeces, where smaller samples were also used. From the results of the latter two substances, it seems that the problem of heterogeneity of the samples can be overcome by carrying out repeat analyses on the same batch of material.

The linearity of the measurements performed on the emission spectrometer was determined using ^{15}N-labelled $(NH_4)_2SO_4$ standards (obtained from Isotope Services, Los Alamos National Laboratory, Los Alamos, NM, USA).

Table 3 shows the excellent agreement obtained between the nominal values of the standards and those measured with the automatic emission spectrometer. A correlation coefficient of 0.9996 was found, with a y-intercept of -0.061. The slight deviation from a straight line with a y-intercept = 0 could be attributed to the fact that a higher degree of accuracy can be obtained with a mass spectrometer.

The memory effect of the automatic ^{15}N analyser is not

Table 1. Accuracy and reproducibility of nitrogen analyses performed using the automatic nitrogen analyser

Sample	Mass/ mg	Atom-% of ^{15}N Measured	Mean	Expected	SD	CV
$(NH_4)_2SO_4$	5.07	21.29	21.15	21.10	0.094	0.44
	5.04	21.20				
	5.05	21.15				
	5.05	21.17				
	5.00	21.08				
	5.00	21.02				
Haemin	10.07	8.49	8.51	8.59	0.025	0.29
	10.05	8.53				
	10.05	8.53				
	10.00	8.48				
	10.05	8.53				
	10.06	8.54				
Albumin	14.20	14.96	15.09	15.10	0.121	0.79
	14.19	14.96				
	14.23	15.19				
	14.22	15.20				
	14.19	15.04				
	14.24	15.21				

Table 2. Determination of atom-% ^{15}N in biological samples using Kjeldahl procedures and the automatic nitrogen analyser

Sample	Mean mass/ mg	Atom-% N in sample Kjeldahl	ANA	SD	CV
Bacteria	2.04	6.66	6.66	0.136	2.0
Maize cell walls	6.51	0.14	0.18	0.004	2.27
Duodenal contents	2.04	3.30	3.85	0.105	2.74
	10.02	3.30	4.09	0.064	1.57
Sheep faeces	2.02	2.51	2.41	0.038	1.59
	10.04	2.51	2.51	0.056	2.23

Table 3. Measured ^{15}N atom-% in a range of ^{15}N standards. Standards obtained from Isotope Services, Los Alamos National Laboratory, Los Alamos, NM, USA

	Measured atom-% ^{15}N		
Nominal atom-% ^{15}N	$\bar{X}$ ($n = 6$)	SD	CV
0.37	0.358	0.006	1.68
0.5	0.481	0.018	3.74
1.0	0.898	0.022	2.45
2.0	1.764	0.025	1.42
5.0	4.528	0.081	1.79
10.0	9.521	0.118	1.24

Table 4. Test of the memory effect in the discharge tube of the ADSRI nitrogen-15 analyser

	Atom-% ^{15}N		
Sample No.	Nominal	Measured	Average
1	0.365	0.357	
2	0.365	0.373	
3	0.365	0.391	0.364 ± 0.019
4	0.365	0.342	
5	0.365	0.356	
6	16.603	16.819	
7	16.603	16.723	
8	16.603	16.752	16.806 ± 0.133
9	16.603	16.705	
10	16.603	17.030	
11	0.365	0.369	
12	0.365	0.362	
13	0.365	0.371	0.368 ± 0.006
14	0.365	0.376	
15	0.365	0.361	

significant and can be ignored. The results in Table 4 show that even under extreme conditions, where the highly enriched samples were immediately followed by a sample at natural abundance, no significant increase could be detected. Goulden and Salter[16] also found no memory effect when they subjected the NIRD automatic analyser to a highly enriched sample followed by one at natural abundance.

The most important feature of the automatic nitrogen-15 analyser is the fast rate of analysis possible (12–20 samples per hour compared with 6–8 samples per week with manually operated emission spectrometers[12]). It is also more advanced than the automatic system described by Goulden and Salter,[16] as biological samples can be analysed directly without prior Kjeldahl digestion and subsequent recovery of ammonium chloride. Further, the system is fully automated with a direct printout of atom-% ^{15}N for each sample.

Although mass spectrometry remains the most accurate means of analysing for nitrogen-15, with a reproducibility of 0.001 atom-% possible in modern apparatus, automatic emission spectrometry offers a number of advantages. The most important are the much faster rate of analysis possible and the very small amount of nitrogen (10 μg) required for accurate determinations, compared with the 200–2000 μg required for determination by mass spectrometer. The automatic nitrogen-15 analyser described in this paper is therefore ideally suited for biological research where metabolites are often only available in small amounts.

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Application of Electrothermal Atomic Absorption Spectrometry to the Determination of Trace Amounts of Indium in Metallic Zinc and Lead

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Trace amounts of indium in metallic zinc and lead have been determined by graphite furnace atomic absorption spectrometry. The detection limits for three operated furnace systems were evaluated: for SP9-01 (1), 6.0×10^{-13} g; for HGA-500 (2), 7.0×10^{-13} g; and for GRM-1268 (3), 1.1×10^{-11} g, each using 20 μl of sample. Linear calibration graphs were obtained between 0.01 and 0.1 p.p.m. (1), 0.05 and 0.6 p.p.m. (2) and 0.05 and 0.2 p.p.m. (3). Ion-exchange separation employing Xylenol Orange modified Amberlyst A-26 anion exchanger was used as the preliminary step for the determination of indium in Zn - In and Pb - In alloys as interferences from other metals were observed in the indium absorbance. In order to compare the results, two other separation methods for the lead matrix and several different graphite furnace atomisers were used. Molar excesses of Ni, Co, Fe, Cu and Zn of less than 1000 and between a 100 and 1000 molar excess of Al cause a decrease in the indium absorbance. With molar ratios greater than 1000, the suppression caused by Al, Co and Zn disappears and that of Fe becomes less pronounced. Ga causes an enhancement of the indium atomic absorption signal. Equivalent amounts of As, Sb, Bi do not interfere.

Keywords: *Indium determination; electrothermal atomisation; atomic absorption spectrometry; ion-exchange separation; Xylenol Orange modified Amberlyst A-26*

Indium is frequently found in trace amounts in metallic zinc and lead. Its determination in metals and ores (in nature indium occurs in some zinc and lead ores) is important from an analytical point of view. The determination of indium, especially in the presence of the other metal matrices, presents many problems for the analytical chemist. The aim of the work reported in this paper was to determine trace amounts of indium in metallic zinc and lead using atomic absorption spectrometry (AAS) with electrothermal atomisation (ETA). AAS was chosen for indium determination as the most convenient means of comparing the results with other methods of separation. In preliminary experiments we found that there were interferences from matrix metals. Preliminary separation of indium from matrix metals was adopted as the most practical procedure to avoid matrix interferences. Three independent methods of separation were investigated: co-precipitation of hydroxides, precipitation of lead sulphate and ion-exchange separation. We found ion-exchange separation on Xylenol Orange modified anion exchanger to be the most convenient method.

Experimental

Instrumentation

A Pye Unicam SP9-01 atomic absorption spectrometer, a Perkin-Elmer Model 2380 atomic absorption spectrometer equipped with an HGA-500 graphite furnace atomiser and a Beckman Model 1272D spectrophotometer equipped with an Unilam 1288 burner and a Pye Unicam GRM-1268 graphite furnace atomiser were used. The three instrument systems were equipped with deuterium background correction. The deuterium lamp background compensator could not eliminate all the matrix interferences observed. The operating conditions are given in Table 1. The SP9-01 atomic absorption spectrometer was used for the determination of indium in all the metallic lead and zinc samples. For the examination of the indium content in the weighed samples and for comparison of results indium was also determined using the Beckman 1272D spectrophotometer and the GRM-1268 graphite furnace atomiser.

Table 1. Optimum operating conditions for the determination of indium by ETA atomic absorption spectrometry

Parameter	Pye Unicam SP9-01	HGA-500 (Perkin-Elmer 2380)	Pye Unicam GRM-1268 (Beckman 1272A)
Wavelength/nm	303.9	303.9	303.9
Drying temperature/°C	110	100	100
Drying time/s	15	20	20
Charring temperature/°C	500*	1200	1100–1350
Charring time/s	15	20	30
Atomisation temperature/°C	2400	2500	2700–2900
Atomisation time/s	15	10	10
Cleaning temperature/°C	3000	2500	3000
Cleaning time/s	5	3	3
Band-pass/nm	0.7	0.7	0.7
Injection volume/μl	20	20	20
Argon flow-rate/l min^{-1}	2	Standard (0.3)	Standard (1.5)

* Explanation in text.

Reagents

A standard indium solution was prepared by dissolving indium (spectral grade, 1.000 g) in 2 M HCl and diluting to 500 ml. This solution was further diluted as required.

Metal ion solutions were generally prepared by dissolving the appropriate masses of the sulphates and nitrates of the metals in doubly distilled water and were standardised by EDTA titration. The stock solutions were diluted as required.

Ion-exchange Columns

Columns 25 cm long and 5 mm i.d., with a stopcock at the end, were used. They were packed with the macroporous strong base anion-exchange resin Amberlyst A-26, with a bead size of 0.1–0.2 mm (Rohm and Haas), modified by the use of Xylenol Orange (XO), tetrasodium salt.[1] In procedures A and B, in order to obtain XO-loaded resin, the Amberlyst A-26 chloride form with a bead size of 0.1–0.2 mm was shaken with a 4×10^{-5} M aqueous solution of XO until the supernatant became colourless. The resin was then filtered off, washed with water and ethanol, dried and stored in a refrigerator.[1] The modified resin had a capacity of 0.4 mmol of XO per gram of Amberlyst A-26 in the primary chloride form. A 4 cm bed height of 200 mg of modified resin was used in both procedures. Flow-rates of 40 ml h^{-1} were used.

Ion-exchange Separation of Indium from Zinc and Lead

Preliminary experiments confirmed the usefulness of XO-loaded resin for the separation of indium from zinc and lead. To optimise the conditions for the separation of trace amounts of indium from the great excess of lead and zinc, some investigations were performed with the use of synthetic solutions simulating the composition of the real metallic zinc and lead samples being analysed. The effect of bed height and the size of the resin beads was investigated. The following procedures for the rapid separation of indium from zinc (A) and from lead (B) were deduced.

Procedure A

A 13-ml volume of solution containing 0.005 mg of In and 669 mg of Zn adjusted to pH 2.0 was introduced into the column. Zn was eluted with 25 ml of water acidified to pH 4.0 with H_2SO_4. Indium was eluted with 20 ml of 0.2 M HNO_3 into a 25-ml calibrated flask. After dilution to volume, indium was determined according to the conditions given in Table 1. The indium concentration was obtained from a calibration graph. The mean obtained from six separate determinations was $0.0049 \pm 1.3 \times 10^{-4}$ mg (95% confidence limit). Each result was the mean of four AAS measurements by the GRM-1268. The mean obtained using the Pye Unicam SP9-01 spectrophotomer was $0.0048 \pm 1.2 \times 10^{-4}$ mg (95% confidence limit).

Procedure B

A 13-ml volume of solution containing 0.005 mg of In, 669 mg of Pb and 2 mg of XO adjusted to pH 3.0–3.8 was introduced into the column. Pb was not retained on the column and passed into the eluent. To wash the Pb from the void volume, 25 ml of water acidified to pH 4.0 with HNO_3 were used. Indium was eluted with 20 ml of 0.2 M HNO_3 into a 25-ml calibrated flask. The volume was adjusted to the mark and indium was determined by AAS. The indium concentration was obtained from the calibration graph. The mean obtained from six separate determinations was $0.0049 \pm 1.3 \times 10^{-4}$ mg (95% confidence limit). Each result was the mean of four AAS measurements by the GRM-1268. The mean obtained using the Pye Unicam SP9-01 spectrometer was $0.0049 \pm 1.2 \times 10^{-4}$ mg (95% confidence limit).

Analysis of Metallic Zinc, Procedure 1, and Metallic Lead, Procedures 2, 3 and 4

The sample of metallic zinc was dissolved by heating it with 5 ml of HNO_3 (1 + 1). The solution was evaporated twice, and the residue was dissolved in 0.2 M HNO_3, transferred into a 25-ml calibrated flask and diluted to volume. Five different samples of metallic lead were dissolved in the same way and transferred into five 25-ml calibrated flasks.

Procedure 1

A 5-ml aliquot was diluted to 10 ml, adjusted to pH 2.0 and introduced into the ion-exchange column. Zn was not retained on the resin. Zinc remaining in the void volume was washed out with 25 ml of water acidified to pH 4.0 with H_2SO_4. Indium was then eluted with 20 ml of 0.2 M HNO_3 into a 25-ml calibrated flask and was diluted to volume. The indium was determined by AAS according to the conditions given in Table 1. The concentration was obtained from the calibration graph, and the results of the analysis are presented in Table 2.

Procedure 2

A 5-ml aliquot was diluted to 10 ml and adjusted to pH 3.0–3.8; 2 mg of XO were then added and the aliquot was introduced into the column. Pb was not retained on the resin bed and passed into the eluent. Lead was washed out with 25 ml of water acidified to pH 4.0 with HNO_3. Indium was eluted with 20 ml of 0.2 M HNO_3 into a 25-ml calibrated flask. The solution was diluted to volume and indium determined by AAS; the concentration was obtained from the calibration graph. The results are presented in Table 3.

Procedure 3

Five samples of metallic lead containing different amounts of indium were analysed. They were dissolved by heating with 5 ml of nitric acid (1 + 1) and then 20 mg of metallic iron, dissolved in 1 + 1 nitric acid, were added to all the samples and metal hydroxides were coprecipitated with ammonia solution.

Table 2. Determination of indium in metallic zinc by ETA - AAS

	Direct determination*		Determination after ion-exchange separation† (Procedure 1)	
Instrument	Sample/mg	In, %	Sample/mg	In %
GRM-1268 . .	1020	0.0009	1125	0.0005 ± 0.0002
SP9-01	1005	0.0009	1015	0.0006 ± 0.0002

* Obtained from 10 AAS measurements without zinc separation, after dissolving the sample as described before Procedure 1.
† Mean and range (95% confidence limit) of four separate determinations.

Table 3. Determination of indium by ETA - AAS in five different samples of metallic lead after the separation step obtained for the GRM-1268 (*a*) and SP9-01 (*b*). All samples analysed contained a mixture of metal ions (Cu, Ag, Bi, As, Sb, Sn, Tl) in the range 0.045–0.0006% for each metal ion

Sample No.	Separation by coprecipitation of hydroxides (Procedure 3)		Separation by precipitation of lead sulphate (Procedure 4)		Ion-exchange separation (Procedure 2)	
	Sample/mg	In,* %	Sample/mg	In,* %	Sample/mg	In,† %
1(*a*)	544.7	0.028	512.1	0.020	532.0	0.025 ± 0.001
1(*b*)	512.0	0.025	543.2	0.020	508.1	0.024 ± 0.002
2(*a*)	1126	0.012	1140	0.013	1012	0.015 ± 0.004
2(*b*)	1108	0.011	1114	0.011	1007	0.012 ± 0.003
3(*a*)	933.8	0.002	1002	0.001	1104	0.0018 ± 0.0003
3(*b*)	921.4	0.003	1011	0.002	1103	0.002 ± 0.0004
4(*a*)	1010	0.0007	1115	0.0006	1205	0.0007 ± 0.0003
4(*b*)	1013	0.0006	1112	0.0005	1201	0.0006 ± 0.0003
5(*a*)	1103	0.008	1232	0.007	1125	0.0070 ± 0.0018
5(*b*)	1105	0.008	1114	0.007	1112	0.0070 ± 0.0021

* Obtained from four AAS measurements for one sample of metallic lead, after one separation step.
† Mean and range (95% confidence limits) for four AAS measurements in each of four separate determinations.

This was repeated twice. The hydroxide precipitate was dissolved in 5 ml of HNO_3 (1 + 1), transferred into a 25-ml calibrated flask and diluted to volume. Indium was determined by AAS according to the conditions given in Table 1. The results were corrected for blanks determined under the same conditions as the lead samples.

Procedure 4

For the comparison of results, the lead matrix in all alloy samples was separated as lead sulphate, after the dissolution step described above, and indium was determined in solution after filtering off the precipitate. For this, 5 ml of concentrated H_2SO_4 were added to the aliquot and the sample was heated until white fumes were observed. The sample was then cooled and, after adding 50 ml of water, heated to boiling. In this instance, the indium standards were mixed with lead nitrate and then analysed in exactly the same way. The results obtained are presented in Table 3.

Results and Discussion

Results were obtained by three different ETA - AAS systems. The Beckman 1272D system belongs to an earlier generation of spectrophotometers, but is still used by many laboratories. The results obtained using it are comparable with those from a newer type of spectrophotometer, the Pye Unicam SP9-01. This implies that even those laboratories that possess older types of spectrophotometers may use our method with good results.

To optimise the charring temperature, the dependence of absorbance on charring temperature was investigated (Fig. 1). A charring temperature of 1200 °C applied for 20 s was the maximum temperature at which no loss of analyte occurred when the HGA-500 was used. For the SP9-01 spectrometer, a charring temperature of 500 °C applied for 15 s was advised by Pye Unicam for indium determination. There was no difference in the absorption peak at charring temperatures in the range 500–1000 °C in the determination of indium. For the GRM-1268, an optimum charring temperature of 1100 °C applied for 20 s was chosen.

Atomisation temperature *versus* absorbance is presented in Fig. 2. The maximum temperatures available using the HGA-500, 2400 °C for the SP9-01 and 2700–2900 °C for the GRM-1268, were chosen. These maximum temperatures were also recommended by Dittrich.[2,3] As expected, the best analytical results were obtained using the HGA-500 and SP9-01 systems. For the GRM-1268, L'vov's pyrolytic graphite platform was used, but no improvement was obtained. The

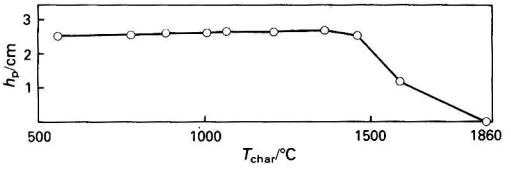

Fig. 1. Height of indium absorption peak as a function of charring temperature. GRM-1268, $T_{atom.}$ = 2800 °C, c_{In} = 0.1 p.p.m., $t_{char.}$ = 30 s

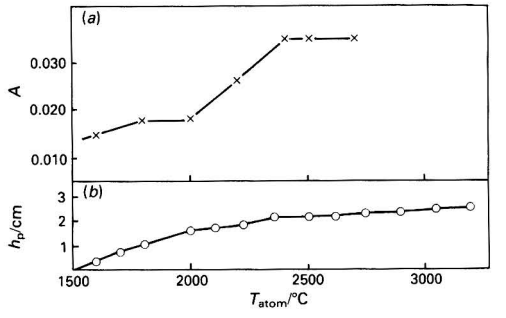

Fig. 2. (*a*) Absorbance of indium; and (*b*) peak height as a function of atomisation temperature. (*a*), HGA-500, $T_{char.}$ = 1200 °C, c_{In} = 0.4 p.p.m. (*b*), GRM-1268, $T_{char.}$ = 1100 °C, c_{In} = 0.1 p.p.m.

detection limit with the GRM-1268 is similar to[2,4] or better than[3] those in the literature. The presence of NaOH, HCl or HNO_3 has a strong influence on the determination of indium, as has also been observed by others.[2,4,5] According to Dittrich,[2,3] the suppression of the indium absorbance in the presence of HCl is due to InCl formation. We think that a more probable explanation for the decrease in the indium signal is the diffusion of volatile $InCl_3$ from the furnace in the period of time before the atomisation temperature has been reached. The presence of HCl and NaOH causes a proportional suppression of absorbance, whereas the addition of HNO_3 (>0.18 M) stabilises the signal. Hence, 0.2 M HNO_3 was chosen as the best medium for the determination of indium.

Linear calibration graphs were observed between 0 and 0.6 p.p.m. for the HGA-500, between 0 and 0.2 p.p.m. for the GRM-1268 and between 0 and 0.1 p.p.m. for the SP9-01. In all instances a 20-μl aliquot was used.

Table 4. Detection limit and sensitivity (for 0.0044 absorbance) for the determination of indium with different spectrophotometers. In all instances indium was determined under the optimum conditions given in Table 1; 20-μl volumes were used

Parameter	Pye Unicam SP9-01	Perkin-Elmer 2380 (HGA-500)	Beckman 1272D (Pye Unicam GRM-1268)
Detection limit/ng ml^{-1} . .	0.030	0.035	0.55
Detection limit/pg	0.60	0.70	11
Sensitivity/ng ml^{-1}	0.050	0.050	2.2
Sensitivity/pg	1.0	1.0	44
Reproducibility/ng ml^{-1} . .	±0.0052	±0.0052	±0.10

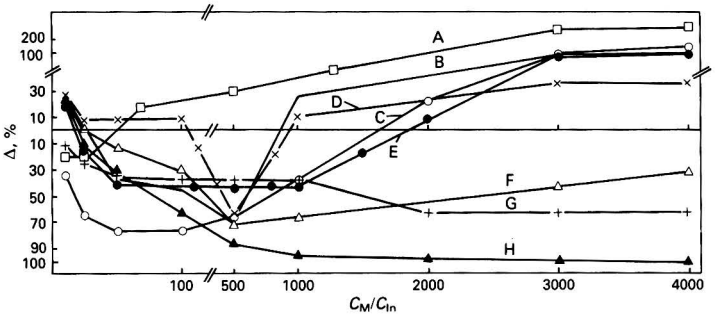

Fig. 3. Effect of Al, Fe, Co, Ni, Cu, Zn, Ga and Pb on the AAS signal of In, expressed as relative change in signal, for the HGA-500 and GRM-1268 systems. A, Ga; B, Co; C, Fe; D, Al; E, Zn; F, Ni; G, Pb; and H, Cu

Zinc, lead and other matrix metals have a marked effect on the indium absorbance; metals were taken as nitrates throughout to avoid the influence of chlorides. Molar excesses of Ni, Co, Fe, Cu and Zn of less than 1000 and of Al between 100 and 1000 cause a decrease in the indium absorbance. With molar ratios greater than 1000, the suppression caused by Al, Co and Zn disappears, and that of Fe becomes less pronounced. Gallium causes an enhacement of the indium atomic absorption signal. These results were obtained by the HGA-500 system and by the GRM-1268 system for comparison, and are presented in Fig. 3. Equivalent amounts of As, Sb and Bi do not interfere with the indium atomic absorption signal.

The complex nature of the chemical process taking place in the graphite furnace makes the correct explanation of the interference effects observed extremely difficult. Campbell and Ottaway[6] and Aggett and Sprott[7] have postulated that atoms are produced by direct reduction of the metal oxides by graphite. If this is true, the suppression of the indium absorbance in the presence of an excess of the other metals, especially of those forming more stable carbides or refractory oxides, may be explained simply by the occlusion of the indium or the graphite surface. Under such conditions the absorption peak of indium was observed to be delayed and was of a different shape. We observed the suppression of the indium absorption signal in the presence of Fe, Co and Ni, which form carbides with high melting-points, and in the presence of Al (refractory oxide), Zn (high sublimation point), Pb and Cu. A strong influence of the presence of zinc and lead on the absorption signal of indium was observed; this was the reason for developing Procedures 1, 2, 3 and 4. Some modification of the charring step by an extension of time could not remove the interference of lead and zinc on the indium signal. Magnesium nitrate was investigated as a matrix modifier, but it did not remove the interferences. $Mg(NO_3)_2$ causes an enhacement of the indium atomic absorption signal related to its concentration in the sample.

If the excess of interfering metal is sufficiently large, a new effect is observed: an increase of the indium signal. This also suggests that the mechanism of atomisation may have been changed. This effect is very similar to that observed if a modified graphite furnace (modified by means of some metal ions forming stable carbides or other compounds) is used for the atomisation process.[8-10] No presence of indium in interfering reagents, even at high interfering metal concentrations, was reported.

Owing to the complicated effects on the indium signal resulting from the presence of other metal ions, we decided to eliminate matrix interferences by a preliminary separation of indium from the sample of metallic zinc and lead by ion exchange.

The methods were applied to the determination of indium in metallic zinc and lead. The results are presented in Tables 2 and 3. In order to compare the determination in metallic lead, we also used other separation methods, namely, coprecipitation and precipitation of the matrix by the use of H_2SO_4. In the coprecipitation method, indium, as $In(OH)_3$, coprecipitates on $Fe(OH)_3$. This method was also adopted for the separation of indium. The results are presented in Table 3. The XO-loaded resin permits the simple, fast separation of indium from an excess of lead and zinc using a very short resin bed. No influence from other metal ions present in up to a 10-fold molar excess was observed.

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Determination of Arsenic(V) in Aqueous Solutions by D.c. Argon Plasma Emission Spectrometry. Interference Studies

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The linear dynamic range, detection limits, accuracy and precision of the determination of As(V) in aqueous solutions by d.c. argon plasma emission spectrometry were studied at four emission lines of arsenic(V). The interference and matrix effects of eight common acids and 12 cations [(Na(I), K(I), Mg(II), Ca(II), Al(III), Cr(III), Cr(VI), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II)] on the determination of arsenic(V) in aqueous solutions were investigated at the arsenic emission line at 193.696 nm. The linear range covered concentrations from 0.05 to 100 μg ml^{-1} of As(V). The RSD increased from 0.5 to 5% when the concentration of As(V) decreased from 10 to 0.75 μg ml^{-1} and from 15 to 50% in the range 0.5–0.05 μg ml^{-1} of As(V). The minimum detection limit was 0.063 μg ml^{-1}, calculated to 3σ of ten measurements of blank, as recommended by IUPAC.

Keywords: *Arsenic(V) determination; plasma emission spectrometry; interference studies; matrix effects*

Arsenic and its compounds are extremely toxic and concern about their levels in the environment has stimulated great interest in the development of analytical methods for the determination of arsenic in a variety of sample matrices.

Several different plasma emission methods for the determination of arsenic have been investigated by various workers. These include the direct determination of arsenic by direct current plasma emission spectrometry (DCP-AES)[1,2,3] and inductively coupled plasma emission spectrometry (ICP-AES),[4–7] and determination by ICP-AES[8–10] with hydride generation systems (HY-ICP-AES), microwave-induced plasma emission spectrometry and DCP-AES with hydride generation systems (HY-MIP-AES[11,12] and HY-DCP-AES[3]). Extensive investigation of hydride-forming elements have been carried out by Thompson *et al.*[8,9] using HY-ICP-AES. Simultaneous multi-element determinations including arsenic have been made by ICP-AES[4,6,7] and by HY-ICP-AES.[8,9,13] In comparison with the ICP-AES technique and the methods relying on hydride generation, the direct determination of arsenic by DCP-AES has been relatively little studied.

Experimental

Instrumentation

A Spectrametric SpectraSpan III single-channel plasma emission spectrometer with a d.c. argon plasma source and an échelle monochromator with an average resolution of 0.003 nm was used for the emission measurements. Data output was via a Hewlett-Packard 85A processor.

The slit openings, as recommended by the manufacturer, had an entrance of 50 × 300 μm and an exit of 100 × 300 μm. The photomultiplier voltage used was 750 V. The argon gas flowed through the electrode sleeves and nebuliser at 50 and 30 lb in^{-2}, respectively. The solution uptake rate was maintained at 1.6 ml min^{-1} by a peristaltic pump. An integration period of 3 s was used, with three integrations per sample and standard.

The spatial profile of the plasma was peaked horizontally and vertically. The optimum position in the plasma source, where the maximum net signal to background ratio is obtained, was at approximately 0.2 mm below the intersection of the three plasma legs when the plasma was at about the "−1" position specified in the manual.[14]

Reagents

All reagents were of analytical-reagent grade. A standard stock solution of arsenic (2000 μg ml^{-1}) was prepared by diluting an ampoule of As_2O_5 with water (Merck, Titrisol). Except for HBr, the strong acids (Merck) were diluted to 10 M solutions before being used to prepare the solutions used in the investigation.

The water was distilled and de-ionised immediately before use (Milli Q system). All glassware was acid-washed before use.

Results and Discussion

Instrumental Parameters

The most common emission line for the determination of arsenic by plasma emission spectrometry is 193.696 nm.[4–7,15] This is also the strongest line in ICP-AES,[16] although many other lines can and have been used.[16,17] For example, Thompson *et al.*[9] used the arsenic lines at 228.812 and 234. 984 nm for their investigation of hydride-forming elements by HY-ICP-AES. Barnett *et al.*[12] selected the line at 234.984 nm for their measurement with a miniature HY-MIP-AES. Degner[5] investigated seven emission lines of arsenic, but he reports only the detection limits, not the intensities. Urasa[2] determined arsenite and arsenate by DCP-AES using the line at 197.197 nm, and Panaro and Krull[3] used the line at 228.812 nm with HY-DCP-AES. Important emission wavelengths and their relative intensities are tabulated in Table 1.

We selected four emission lines for the investigation of arsenic(V), namely, 193.696, 197.197, 199.048 and 228.812 nm (Fig. 1). All of these were expected to be relatively free from direct spectral overlap by foreign elements.

Table 1. Relative intensities of common analytical wavelengths for arsenic with different plasma emission methods

Wavelength/ nm	Relative intensity			
	ICP	HY-ICP	HY-MIP	DCP
193.696 I	145	—	—	83
197.197 I	108	—	—	111
198.970 I	43	—	—	—
199.048 I	—	—	—	67
200.334 I	77	—	—	—
228.812 I	100	100	100	100
234.984 I	100	51	186	—
245.653 I	—	—	23	—
Reference	16	9	12	This paper

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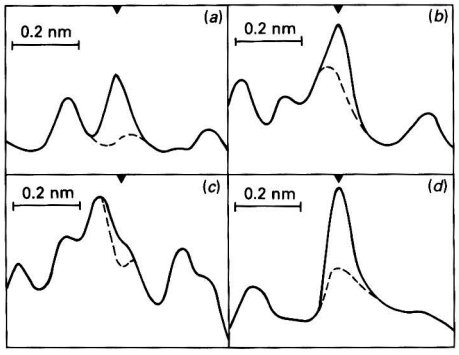

Fig. 1. Spectral profiles of selected arsenic emission lines. (——), As(V) in water (2.5 μg ml^{-1}); (– – –), water. (*a*) λ = 193.696 nm; (*b*) λ = 197.197 nm; (*c*) λ = 199.048 nm; and (*d*) λ = 228.812 nm

Table 2. Detection limits (DL), calculated to 3σ of ten measurements of blank as recommended by IUPAC,[19] background equivalent concentrations (BEC) and relative sensitivity for arsenic emission lines measured by DCP-AES.

Wavelength/ nm	DL/ μg ml^{-1}	BEC/ μg ml^{-1}	Relative sensitivity
193.696	0.063	3.9	83
197.197	0.295	12.1	111
199.048	0.441	21.9	67
228.812	0.117	10.1	100

Detection Limit

The detection limits (DL) of different wavelengths and methods differ appreciably, depending on the analytical line selected, the instrument used, the method of analysis and the form of arsenic determined. Urasa[2] obtained DL of 20 ng ml^{-1} for As(III) and 40 ng ml^{-1} for As(V) using DCP-AES, whereas Panaro and Krull[3] found about 300 ng ml^{-1} for both As(III) and As(V) using the same method.

The hydride generation system is much more sensitive than conventional plasma spectrometry. Oliveira *et al.*[13] have reported DL as low as 1 ng ml^{-1} for As(III) and As(V) by HY-ICP-AES. Very low DL have been obtained by hydride generation system AAS (HY-AAS), *e.g.*, 0.35 ng ml^{-1}.[18]

We determined the detection limit for aqueous solutions of As(V) at the line 193.696 nm using three different methods. The first method was to calculate three times the standard deviation of ten measurements of blanks, as recommended by IUPAC.[19] The second method involved the measurement of the method detection limit for the 99% confidence level[1] and the third was based on the equation DL = 3(RSD) $(I_b/I_a)C_a$, where I_b and I_a are the mean background analyte intensities and RSD the relative standard deviation of n measurements of the analyte at a concentration C_a.[20] The DL values for the different methods were 0.063, 0.088 and 0.079 μg ml^{-1}, respectively. The reproducibility of the detection limits varied from day to day by ±30%, as calculated by the first method.

Calibration Graphs and Precision

The linearity and the analyte concentration range of the As(V) determination was investigated at the four emission lines with standards of 0, 1, 5, 10, 50 and 100 μg ml^{-1}. Not surprisingly, the 197.197 nm line gave the best intensity and sensitivity.[16,17] According to the "Tables of Spectral Line Intensities, Part I,"[17] the line at 199.048 nm should have had better intensity and sensitivity than those recorded here. However, the values in the Tables are based on arc emission spectrometry, and in the corresponding table of Boumans,[16] where the sensitivities are corrected for ICP-AES, the line is one of the weakest.

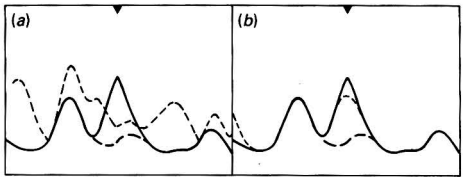

Fig. 2. Spectral profiles of arsenic emission lines at 193.696 nm. (——), As(V) in water (2.5 μg ml^{-1}); (· · · ·), HOAc (*a*) or HF (*b*), both 5 M; and (– – –), water

The calibration graphs of the three most sensitive lines (197.197, 228.812 and 193.696 nm, respectively) are linear from 0 to 100 μg ml^{-1}. The weakest line (199.048 nm) consistently curves upwards. The background equivalent concentrations (BEC) calculated for different concentrations are given in Table 2 together with the sensitivities of the four lines. The emission line at 193.696 nm was selected for more detailed study in this investigation because it had the lowest detection limit and good sensitivity.

The linearity, precision and accuracy of the selected line were studied with three standard series (0.05–2.5, 5–75 and 100–400 μg ml^{-1}). The linear range covered 0.05 to 100 μg ml^{-1}. Above 100 μg ml^{-1}, the deviations from linearity began to increase, although the relative standard deviation values (RSD) were only about 0.5%. The deviations were less than 0.02 and 0.08 μg ml^{-1} in the first and second standard series, respectively. The precision, however, was significantly decreased at the lower concentration levels: the RSD values increased from 0.5 to 5% when the concentration decreased from 10.0 to 0.75 μg ml^{-1} and from 15 to 50% in the range 0.5–0.05 μg ml^{-1}.

Effects of Acid Concentration

In order to avoid errors in the determination of As(V), it is necessary to match the total acid content of the samples and standards as closely as possible. Viscosity changes produced by variations in the total acid content affect sample transport properties and therefore the analytical sensitivities.[7] Other factors, such as aerosol transport losses and changes in the excitation conditions in the plasma, also play a role.[15]

The effects of eight acids (HF, HCl, HBr, HNO_3, $HClO_4$, H_2SO_4, H_3PO_4 and HOAc) on determinations of 2.5 μg ml^{-1} As(V) solutions were studied with pure acids at concentrations of up to 5 M. Only two of the acids has a significant background effect. HF showed an emission line at the same wavelength as As(V) [Fig. 2(b)], and HOAc gave many lines, as can be seen in Fig. 2(*a*), and the background level was higher than with other acids. These two acids caused the largest signal enhancements of As(V); they increased the intensity of the As line in an almost linear manner in up to 5 M acid solutions, in which the intensities were +60 and +110% higher, respectively, than in an aqueous solution. A linear correlation between intensity and acid concentration was also observed for HBr, the increase being +30% in 5 M acid solutions (Fig. 3).

In 0.1 M H_2SO_4 and 0.5 M $HClO_4$ solutions the intensity was decreased by about −4 and −10%, respectively, from where it decreased linearly with increasing acid concentration to −30% for H_2SO_4 and remained approximately constant for $HClO_4$. The H_3PO_4 solutions initially increased the intensity slightly, with a maximum at about 1.5 M, and then there was a linear decrease to −18%. The deviations in HCl and HNO_3 solutions remained approximately constant at about −2 to −5% lower than in water solutions.

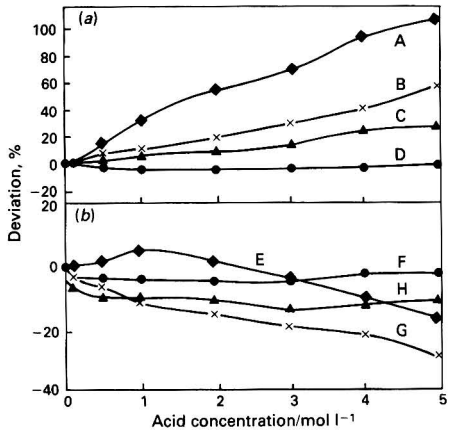

Fig. 3. Effect of different acids on 2.5 μg ml^{-1} of As(V) in water at the emission line at 193.696 nm. (*a*) A, HOAc; B, HF; C, HBr; and D, HCl. (*b*) E, H_3PO_4; F, HNO_3; G, H_2SO_4; and H, $HClO_4$

Our results are in agreement with the slight suppressing effect of HNO_3 and HCl and the very strong suppressing effect of H_2SO_4 reported for the determination of As by ICP-AES,[15,21] and also with the acid effect of $HClO_4$ reported in a multi-element determination by ICP-AES.[7]

Effect of Foreign Elements

The 2.5 μg ml^{-1} As(V) solution was spiked with 10–5000 μg ml^{-1} of different nitrate salts [except Cr, which was added as Cr(III) chloride and Cr(VI) oxide]. The cations Mg(II), Ca(II), Al(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) increased the intensity of the As(V) line only slightly: deviations in 1000 μg ml^{-1} solutions were less than 4%, whereas in solutions of 5000 μg ml^{-1}, Na and K caused deviations of less than 5%, Mg, Ca, Ni, Cu and Zn deviations of about 10% and Al, Fe and Co deviations of about 24, 19 and 14%, respectively. The greatest effect was caused by Cr(III) chloride and Cr(VI) oxide, both of which enhanced the intensity linearly up to 500 μg ml^{-1}, where the deviations were about 14 and 8%, respectively. In 1000 and 5000 μg ml^{-1} solutions the intensities were 23, 81 and 15, 61% stronger, respectively (Figs. 4 and 5).

Literature values for the spectral interference effects of 1000 μg ml^{-1} of Al as determined by ICP-AES are larger than the values in this study.[6,7] according to Tao *et al.*,[6] 1000 μg ml^{-1} of Al are equivalent to 23.9 μg ml^{-1} of arsenic at the emission line 193.696 nm, however, we observed only a slight increase in deviation with 1000 μg ml^{-1} and a +24% deviation with 5000 μg ml^{-1} of Al at the same wavelength. Other elements reported to cause an interference effect are Fe, Mg and Ti. Solutions of 1000 μg ml^{-1} of these elements were found to be equivalent to 2.92, 0.206 and 0.400 μg ml^{-1} of As, respectively.[6]

Degner[5] investigated the interference effects of Fe, Cr and Cu at seven different emission lines of arsenic by ICP-AES. The maximum concentrations of these elements that caused no interference effects were 10, 100 and 1000 μg ml^{-1} for Fe, Cr and Cu, respectively. In our study, the deviations of arsenic with 1000 μg ml^{-1} of Fe, Cr(III) and Cr(VI) added were about +4, +23 and +15 μg ml^{-1}, whereas no effect was found with Cu addition.

Elements such as Ca, Mg, Na and K often cause a stray light effect in ICP-AES[6] and DCP-AES.[1,22,23] We observed a deviation of only about 3% for 1000 μg ml^{-1} solutions, and of about +10 and +5% deviation for 5000 μg ml^{-1} solutions of Ca, Mg and Na, K, respectively.

The foreign elements have less interference effects in our study by DCP-AES than in the reported studies by ICP-AES. This is due to the very good resolution of the échelle monochromator that provides a two-dimensional spectral pattern with an average resolution of 0.003 nm.[14]

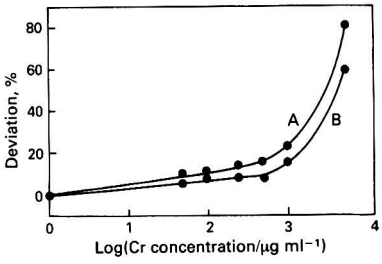

Fig. 4. Effect of Cr(III) and Cr(VI) on 2.5 μg ml^{-1} of As(V) in water at the emission line at 193.696 nm. A, Cr(III); and B, Cr(VI)

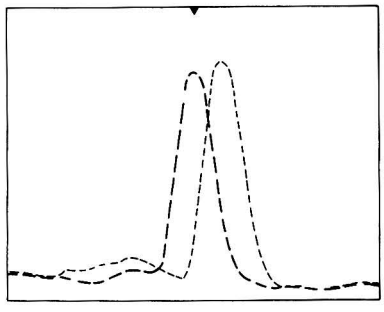

Fig. 5. Spectral profiles at 193.696 nm of 2.5 μg ml^{-1} As(V) in water (– – –) and of 5000 μg ml^{-1} Cr(VI) in water (· · ·) after subtracting the signal due to water

Conclusions

The As(V) emission line of 193.696 nm gave the lowest background and detection limit and good sensitivity. The acids HOAc and HF caused strong spectral interference and increased intensities the most; HCl and HNO_3 had the least effect. Among the cations, Cr(III) and Cr(VI) had the greatest enhancing effects. The stray light effects of Ca, Mg, Na and K, and the matrix effects of Al, Fe, Co, Ni, Cu and Zn, were almost non-existent at 1000 μg ml^{-1} concentrations.

The precision decreased significantly below As(V) concentrations of 0.75 μg ml^{-1}, which limits the usefulness of the direct method. Sensitivities are considerably better for environmental samples using the hydride generation system in AAS and AES. At high concentrations of arsenic the DCP-AES method without hydride generation has the advantage of allowing the simultaneous determination of other elements.

We express our thanks to Professor Heikki Hyvärinen of the Department of Biology, University of Joensuu, for placing the SpectraSpan III instrument at our disposal.

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Study of Organic Interferences in the Spectrophotometric Determination of Nitrite Using Composite Diazotisation - Coupling Reagents

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A study was made of organic interferences in the spectrophotometric determination of nitrite by the diazotisation - coupling technique using three composite reagents [sulphanilamide and *N*-(1-naphthyl)ethylenediamine (NED); sulphanilic acid and NED; and 4-nitroaniline and NED]. Many organic substances interfere, usually causing low results. The interference is usually less with the 4-nitroaniline - NED and sulphanilamide - NED methods than with the sulphanilic acid - NED method. The interferents tested included aliphatic amines (primary, secondary and tertiary), aromatic amines (primary, secondary and tertiary), various phenolic compounds and miscellaneous organic compounds (sucrose, dextrose, lactic acid, succinic acid, acetamide, acetanilide, ethylenediamine tetraacetate, cholesterol, rennin, dodecyl sodium sulphate, acetophenone, urea, citric acid, caffeine, saccharin, morpholine, L-asparagine, gelatin, benzoic acid, formaldehyde, cinchonine, nicotinic acid, trypsin, creatine, starch, albumin, gum tragacanth, casein, formic acid, sorbic acid, ascorbic acid and acetaldehyde). The effect of detergents and soap was also examined. Large amounts of water-miscible solvents (methanol, ethanol, acetone and glycerin) can be tolerated. Water-immiscible solvents do not affect the colour.

Keywords: *Nitrite determination; organic interferences; composite diazotisation - coupling reagents; spectrophotometry*

The authors have previously formulated conditions for the spectrophotometric determination of nitrite using three composite diazotisation - coupling reagents [sulphanilamide and *N*-(1-naphthyl)ethylenediamine (NED); sulphanilic acid and NED; and 4-nitroaniline and NED].[1] Recently, they have studied inorganic interferences in the methods.[2] The purpose of the work reported here was to study organic interferences. The problem of organic interferences is important because nitrite is frequently determined in the presence of organic materials, as in the characterisation of waters, wastes, food and chemical processes. No comprehensive study of organic interferences in the spectrophotometric determination of nitrite by the diazotisation - coupling technique has been made and even qualitative data are scant.[3,4]

Experimental

Reagents

The organic compounds used in this investigation were purchased from Eastman Kodak or Aldrich Chemical. The detergents and soap were ordinary commercial products.

Standard nitrite solution A, 1 ml = 100 μg *of* NO_2-*N*. Sodium nitrite (0.4926 g) was dissolved in water and diluted to 1 l in a calibrated flask.

Sodium nitrite solution B, 1 ml = 1.00 μg *of* NO_2-*N*. This was prepared fresh daily from standard nitrite solution A.

Sulphanilamide - NED reagent. Sulphanilamide (2.5 g) was dissolved in 650 ml of 1 M hydrochloric acid, 30 ml of 0.20% NED solution were added and the solution was diluted to 1 l.

Sulphanilic acid - NED reagent. Sulphanilic acid (5.00 g) was dissolved in a mixture of 750 ml of water and 35 ml of 1 M hydrochloric acid by heating, the solution was cooled to room temperature, 25 ml of 0.20% NED solution were added and the solution was diluted to 1 l.

4-Nitroaniline - NED reagent. 4-Nitroaniline (2.50 g) was dissolved in 300 ml of sulphuric acid (1 + 1) by heating, the solution was cooled to room temperature, 50 ml of 0.20% NED solution were added and the solution was diluted to 1 l.

The NED solution, sulphanilamide - NED and sulphanilic acid - NED reagents were stored in brown bottles in a refrigerator. The 4-nitroaniline - NED reagent was stored in a brown bottle at room temperature.

Preparation of Calibration Graphs

Appropriate aliquots[1] of standard nitrite solution B were transferred into 50-ml calibrated flasks, 10 ml of composite reagent were added from a graduated cylinder, the volume was diluted to the mark and the solution was mixed. The absorbance was measured against the reagent blank at the following wavelengths after the following times: sulphanilamide - NED, 542 nm and 15 min; sulphanilic acid - NED, 541 nm and 30 min; and 4-nitroaniline - NED, 542 nm and 10 min. The absorbance was plotted against micrograms of NO_2-N per 50 ml.

Study of Organic Interferences

Standard nitrite solution B (5 ml) and water were added to 50-ml calibrated flasks. Various amounts of interferent solution were then added and the solution was allowed to stand for about 10 min. Composite reagent (10 ml) was added, the solution diluted to the mark and the absorbance measured as described under Preparation of Calibration Graphs. After consideration of the many analyses for each interferent for each of the three composite reagents, the tolerance limit for the interferent (the limit beyond which the interferent produced an error greater than 0.015 absorbance unit) and the effect of the interferent beyond the tolerance limit were established.

Results and Discussion

Aliphatic Amines

The results for the interference of aliphatic amines, aromatic amines, phenolic compounds, miscellaneous organic compounds and typical detergents and soap are shown in Table 1.

Aliphatic amines can cause low results with all three reagents. The tolerance limit for the aliphatic amines with the

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Table 1. Tolerance limits for organic interferences. ide - NED = sulphanilamide - NED; ic - NED = sulphanilic acid - NED; 4N - NED = 4-nitroaniline - NED

Interferent	Added as*	Tolerance limit/mg per 50 ml			Effect beyond tolerance limit†		
		ide - NED	ic - NED	4N - NED	ide - NED	ic - NED	4N - NED
Aliphatic amines:							
Methylamine	4% W soln.	100	3	800	Low	Low	Low
Ethylamine	5% W soln.	150	10	900	Low	Low	Low
Dimethylamine	5% W soln.	30	2	900	Low	Low	Low
Diethylamine	5% W soln.	150	5	1500	Low	Low	Low
Trimethylamine	2.4% W soln.	25	10	150	Low	Low	Low
Triethylamine	1% W soln.	100	15	300	Low	Low	Low
Aromatic amines:							
Aniline	1% M soln.	10	5	100	Low	Low	Low
4-Chloroaniline	1% M soln.	10	5	50	Low	Low	Low
2,4-Dichloroaniline	1% M soln.	10	5	100	Low	Low	Low
Anthranilic acid (2-aminobenzoic acid)	0.25% M soln.	1.0	0.3	10	Low	Low	Low
Anthranilamide (2-aminobenzamide)	0.25% M soln.	7.5	2	50	Low	Low	Low
Naphthionic acid (4-amino-l-naphthalenesulphonic acid)	0.25% M soln.	0.2	0.15	0.4	Low	Low	Low
N-Methylaniline	5% M soln.	50	10	200	Low	Low	Low
N,N-Dimethylaniline	5% M soln.	100	10	150	Low	Low	Low
Phenolic compounds:							
Phenol	2% W soln.	400	100	100	Low	Low	Low
Salicylic acid	0.5% M soln.	50	50	75	Low	Low	Low
Resorcinol	0.2% W soln.	1.5	1.5	5	Low	Low	Low
3,4-Xylenol	0.2% M soln.	2	0.5	0.2	Low	Low	Low
Pyrogallol	0.2% W soln.	0.05	0.05	0.05	Low	Low	Low
Carvacrol	0.2% M soln.	3	2	3	Low	Low	Low
Vanillin	0.2% M soln.	10	5	15	Low	Low	Low
1-Naphthol	0.2% M soln.	0.2	0.2	0.2	Low	Low	Low
Miscellaneous organic compounds:							
Sucrose	10% W soln.	3000	2000	3000	—‡	Low	—
Dextrose [(+)-glucose]	10% W soln.	3000	2000	3000	—	Low	—
Lactic acid	10% W soln.	1500	500	2000	Low	Low	Low
Succinic acid	5% W soln.	1500	1500	1500	—	—	—
Acetamide	10% W soln.	1000	1000	1500	Low	Low	Low
Acetanilide	0.5% W soln.	150	150	150	—	—	—
Ethylenediaminetetraacetate (Na_2 salt)	1% W soln.	300	200	300	—	Low	—
Cholesterol	2% W soln.	600	600	600	—	—	—
Rennin	1% W soln.	300	300	300	—	—	—
Dodecyl sodium sulphate	2% W soln.	400	300	400	Low	Low	Low
Acetophenone	Acetophenone	300	300	300	§	§	§
Urea	5% W soln.	500	300	50	Low	Low	Low
Citric acid	5% W soln.	50	30	400	Low	Low	Low
Caffeine	2% W soln.	60	30	200	Low	Low	Low
Saccharin	0.4% W soln.	75	75	75	Low	Low	Low
Morpholine	1% W soln.	50	50	75	Low	Low	Low
L-Asparagine	0.5% W soln.	20	10	50	Low	Low	Low
Gelatin	1% W soln.	15	15	75	Low	Low	Low
Benzoic acid	0.1% W soln.	2.0	1.0	2.5	Low	Low	Low
Formaldehyde	0.5% W soln.	3	3	40	Low	Low	Low
Cinchonine	0.05% W soln.	15	5	2.5	—	High	High
Nicotinic acid	0.1% W soln.	10	10	5	Low	Low	Low
Trypsin	0.02% W soln.	6	6	6	—	—	—
Creatine hydrate	0.1% W soln.	3	3	5	Low	Low	Low
Starch (potato)	0.5% W soln.	12.5	10	25	High (colloid)	High (colloid)	High (colloid)
Albumin (egg)	0.1% W soln.	12.5	10	12.5	High (colloid)	High (colloid)	High (colloid)
Gum tragacanth	0.1% W soln.	5	5	5	High (colloid)	High (colloid)	High (colloid)
Casein	0.1% in 0.01 M KOH	5	5	5	High (colloid)¶	High (colloid)¶	High (colloid)¶
Formic acid	0.05% W soln.	0.02	0.02	0.05	Low	Low	Low
Sorbic acid	0.05% W soln.	0.1	0.1	0.05	Low	Low	Low
Ascorbic acid	0.05% W soln.	0.2	0.1	0.2	Low	Low	Low
Acetaldehyde	0.05% W soln.	0.02	0.02	0.1	Low	Low	Low

Table 1—*continued*

Interferent	Added as*	Tolerance limit/mg per 50 ml			Effect beyond tolerance limit†		
		ide - NED	ic - NED	4N - NED	ide - NED	ic - NED	4N - NED
Detergents and soap:							
"All" detergent (Lever Brothers)	0.1% W soln.	10	10	10	High, low (colloid)	High, low (colloid)	High, low (colloid)
"Sparkleen" detergent (Fisher Scientific)	0.1% W soln.	1‖	1‖	1‖	Low	Low	Low
"Ivory" soap (Proctor and Gamble) . .	0.1% W soln.	0.03‖	0.03‖	0.03‖	High, low (ppt.)	High, low (ppt.)	High, low (ppt.)

* W = Water solution and M = methanolic solution; all solutions *m/V*.

† "Low" refers to a negative absorbance error greater than 0.015 in the presence of 5.00 μg of NO_2-N and "high" refers to a positive absorbance error greater than 0.015 in the presence of 5.00 μg of NO_2-N.

‡ —, The effect beyond the indicated tolerance limit could not be established because of the limited solubility of the interferent in the additive solution.

§ Larger amounts did not affect the colour but caused difficulty because of floating droplets.

¶ Precipitate also formed.

‖ See text.

three reagents increases markedly in the order sulphanilic acid - NED, sulphanilamide - NED and 4-nitroaniline - NED and is surprisingly high for the 4-nitroaniline - NED reagent. In the sulphanilamide - NED and 4-nitroaniline - NED methods (but not the sulphanilic acid - NED method), more primary and secondary aliphatic amine can be tolerated than tertiary aliphatic amine. In all the methods, larger amounts of the ethylamines (primary, secondary and tertiary) can be tolerated than the corresponding methylamines. The interference from aliphatic amines is probably mainly caused by the reaction of the aliphatic amine with the nitrite after the addition of the acidic composite reagent. In neutral solution, primary, secondary and tertiary aliphatic amines would merely form amine nitrite salts with the nitrite.[5] However, in the presence of acid, primary aliphatic amines would react with nitrite to produce nitrogen, secondary aliphatic amines would react to produce nitroso compounds and tertiary aliphatic amines would still only form amine nitrite salts.[5] It would seem from these reactions that tertiary aliphatic amines would interfere less than primary and secondary aliphatic amines but, as indicated above, this does not happen in most instances. It is understandable why the ethylamines (primary, secondary and tertiary) would interfere less than the corresponding methylamines, as the ethylamines contain a lower percentage of nitrogen. The 4-nitroaniline - NED method is clearly the method of choice for the determination of nitrite in the presence of aliphatic amines.

Aromatic Amines

Aromatic amines can cause low results in all three methods and the interference is moderate or strong depending on the particular aromatic amine and the method. The tolerance limit for aromatic amines with the three reagents increases in the order sulphanilic acid - NED, sulphanilamide - NED and 4-nitroaniline - NED. Primary aromatic amines interfere more than secondary and tertiary aromatic amines in all the methods. There is no easy explanation for this. In neutral solution, primary, secondary and tertiary aromatic amines would be expected to form amine nitrite salts with the nitrite (although for the secondary and tertiary aromatic amines this salt formation would be limited by the low solubility of these amines in water). In acidic solution, primary aromatic amines would react with nitrite to form diazonium salts, secondary aromatic amines would react to form *N*-nitroso compounds and tertiary aromatic amines would react to form *p*-nitroso compounds.[5] In the methods, it might be expected that the interfering primary aromatic amines would be diazotised and then coupled with the NED, but this is by no means certain. Aromatic amines could possibly interfere by acting as coupling agents in place of the NED. However, in the 4-nitroaniline - NED and sulphanilamide - NED methods such coupling seems unlikely (it is known that aromatic amines can act as coupling agents only in neutral or very weakly acidic solutions[6]). The 4-nitroaniline - NED method is the method of choice for the determination of nitrite in the presence of primary, secondary and tertiary aromatic amines. The problem of the determination of nitrite in the presence of primary aromatic amines is encountered in the analysis of wastes from industrial diazotisation processes.

Phenolic Compounds

Phenolic compounds can cause low results with all three methods. Phenol shows only moderate interference whereas substituted phenols usually produce strong interference. Generally, the interference from phenolic compounds is less with the 4-nitroaniline - NED and sulphanilamide - NED methods than with the sulphanilic acid - NED method. Probably the most important cause of the interference of phenolic compounds is the nitrosation or oxidation of the phenolic compounds by the nitrite after the addition of the composite reagent. It is known that such reactions readily take place in acidic solutions.[5] As would be expected, the interference is especially strong with a compound such as 1-naphthol that is easily nitrosated or a compound like pyrogallol that is easily oxidised. It is not believed that phenolic compounds interfere by acting as coupling agents in place of the NED (phenolic compounds can act as coupling agents only in neutral or alkaline solution.[6]) Either the 4-nitroaniline - NED or sulphanilamide - NED method is the method of choice for the determination of nitrite in the presence of phenolic compounds.

Miscellaneous Organic Compounds

The extent of the interference from miscellaneous organic compounds is varied. Low results are usually produced by an excess of the interferent. Large amounts (greater than 150 mg per 50 ml) of sucrose, dextrose, lactic acid, succinic acid, acetamide, acetanilide, ethylenediaminetetraacetate (disodium salt), cholesterol, rennin, dodecyl sodium sulphate and acetophenone can be tolerated in all the methods. Large amounts of urea can be tolerated in the sulphanilamide - NED and sulphanilic acid - NED methods and large amounts of

Table 2. Interference of water-miscible solvents. Abbreviations etc. as in Table 1.

	Tolerance limit/ml per 50 ml			Effect beyond tolerance limit		
Solvent	ide - NED	ic - NED	4N - NED	ide - NED	ic - NED	4N - NED
Methanol	5	5	20	High	Low	Low
Ethanol	10	10	20	High	Low	Low
Acetone	10	10	25	High	Low	Low
Glycerin	10	10	20	Low	Low	Low

citric acid and caffeine can be tolerated in the 4-nitroaniline - NED method. Moderate amounts (15–75 mg per 50 ml) of saccharin, morpholine, L-asparagine and gelatin can be tolerated in all the methods. Small amounts (usually several milligrams per 50 ml) of benzoic acid, formaldehyde, cinchonine, nicotinic acid, trypsin, creatine hydrate, starch, albumin, gum tragacanth and casein can be tolerated in all the methods. Trace amounts (in some instances less than 0.1 mg per 50 ml) of formic acid, sorbic acid, ascorbic acid and acetaldehyde can cause low results in all the methods. Formic acid, sorbic acid, ascorbic acid and acetaldehyde are all used as food additives,[7] so the tolerance limits should be of practical interest.

In general, it is believed that the cause of the low results from most of the miscellaneous organic compounds is nitrosation or oxidation of the compounds by the nitrite, particularly after the addition of the composite reagent. However, a reaction between the organic compound and the diazotised aromatic amine of the composite reagent or even a reaction between the organic compound and the dye cannot be ruled out. The conditions and mechanism involved in the oxidation of organic compounds are different from those involved in the oxidation of inorganic compounds. The high results caused by starch, albumin and gum tragacanth are due to the colloidal nature of the solutions of these substances. Reasonably accurate results can be obtained in the presence of several times the recommended limits for these substances by making the absorbance measurements against a blank solution not containing the composite reagent. Casein interferes because of the colloidal nature of its solution and because a precipitate is formed on adding the composite reagent. Satisfactory results cannot be obtained by filtering solutions containing starch, albumin, gum tragacanth and casein. Generally, the 4-nitroaniline - NED and sulphanilamide - NED methods will tolerate greater amounts of the miscellaneous organic compounds than the sulphanilic acid - NED method. However, the interference from urea is much greater with the 4-nitroaniline - NED method than the other two methods (probably because of the greater acidity of the 4-nitroaniline - NED reagent).

Detergents and Soap

The interference from detergents and soap is diverse. As indicated in Table 1, the maximum limit for "All" detergent is 10 mg per 50 ml for all the methods and the maximum limit for "Sparkleen" detergent is 1 mg per 50 ml for all the methods. When 10 mg of "Sparkleen" per 50 ml were present, the recoveries in the sulphanilamide - NED, sulphanilic acid - NED and 4-nitroaniline - NED methods were 91, 22 and 81%, respectively. The maximum limit for "Ivory" soap is 0.03 mg per 50 ml for all the methods. When more than this amount is present, a curdy precipitate (stearic acid) is produced on adding the composite reagent. However, if the precipitate is filtered off after adding the composite reagent, up to 3 mg of the soap can be tolerated. More than 3 mg of the soap causes incomplete colour development in all the methods. Either the 4-nitroaniline - NED or sulphanilamide - NED method is recommended for the determination of nitrite in the presence of detergents and soap.

Solvents

The interference from water-miscible solvents is shown in Table 2. It can be seen that large amounts of water-miscible solvents can be tolerated and the tolerance limit for the solvents tested increased in the order glycerin, methanol, ethanol and acetone. The tolerance limits for water-miscible solvents is greater for the 4-nitroaniline - NED method than the other two methods. The effect of water-immiscible solvents (chloroform, hexane, toluene, ethyl acetate and diethyl ether) was also tested. These solvents were found not to affect the colour or extract it and do not interfere if they are separated from the sample solution by use of a separating funnel before or after the development of the colour.

For all the organic substances tested in this work the recovery of nitrite obtained at the recommended limits for the organic interferents was approximately 95% and the reproducibility at these limits was good. On adding an increasing amount of interferent beyond the recommended limit, the error for the recovery of nitrite tended to increase gradually but the results became somewhat erratic (this would be expected by the nature of the interference). The rate of increase of the error for the recovery of nitrite on adding the increasing amount of interferent varied for the 61 organic substances for the three methods.

Organic Compounds in Distilled Water

We conducted almost all of our work using reagent-grade (Fisher Scientific) distilled water. However, we also performed some work using water that is distilled for the Villanova University general chemistry laboratories by means of a high-capacity still. We found that the absorbance obtained for 5 μg of NO_2-N was consistently lower (by about 0.02 absorbance unit) when using the Villanova distilled water. This necessitated the preparation of a different calibration graph. We believe that the cause of the low results is organic matter, which is not removed in a large-scale distillation. The blank obtained using this water was insignificant and the pH was about the same as that of reagent water, *ca.* 6.5. The water distilled on a large scale did not contain detectable amounts of inorganic materials and boiling it (to remove gases) made no difference in the results for nitrite. The standard permanganate test for organic matter, which consists of acidification with sulphuric acid, addition of potassium permanganate and noting any decrease in colour after a few minutes standing, is not sufficiently sensitive to detect small amounts of organic matter.

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Spectrophotometric Determination of Some Pharmaceutical Carbonyl Compounds Through Oximation and Subsequent Charge-transfer Complexation Reactions

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Two spectrophotometric procedures are presented for the determination of six pharmaceutical carbonyl compounds through charge-transfer complexation of their oximes with either the σ-acceptor iodine or the π-acceptor choranil. The optimum assay conditions and their applicability to the determination of the test compounds in pharmaceutical products are described. The results obtained, which compared favourably with those given by a pharmacopoeial method, illustrate the accuracy, sensitivity and simplicity of the developed procedures.

Keywords: *Pharmaceutical carbonyl compound determination; charge-transfer complexation; oximation; spectrophotometry*

Progesterone, testosterone propionate and nandrolone phenylpropionate are Δ^4-3-ketosteroid drugs in common use in therapeutics as progestational, androgenic and anabolic agents, respectively. A number of methods have been described for their determination including titrimetric,[1] colorimetric,[2–7] spectrophotometric,[8] polarographic[9,10] and chromatographic[11–13] procedures. The pharmacopoeial method of analysis for their injectable solutions uses the isoniazide colorimetric[14] method.

Griseofulvin is a potent antifungal antibiotic; carvone and menthone (constituents of oil of caraway and peppermint, respectively) are used as carminatives and flavouring agents in pharmaceutical preparations. The latter is also used as an antipruritic agent. A review of the assay methods for griseofulvin, carvone and menthone, including titrimetric, spectrophotometric, electrochemical and chromatographic procedures, has been described by the authors,[15] and methods of analysis for carbonyl compounds have been surveyed.[16] Official compendia describe several different procedures for the determination of carbonyl compounds in volatile oils, including the acid - base titrimetric - hydroxylamine methods of the BP[17] and EP[18] and the USP[19] method using the neutral sulphate.

Charge-transfer complexation reactions have been extensively used for the determination of electron-donating basic nitrogenous compounds using as reagents the σ-acceptor iodine or polyhalo or polycyano quinone π-acceptors in organic solvents.[20–29] The introduction of a Schiff's base moiety from the reaction of carbonyl compounds with substituted ammonia or hydrazines was first used by the authors[30] in the determination of corticosteroid drugs through solvent extraction and charge-transfer complexation of their phenylhydrazones. The authors have used the oximation reaction for the indirect titrimetric and/or nitrite - diazo coupling spectrophotometric determination of carbonyl drugs.[7,15] Until now, the analytical use of charge-transfer complexation reactions of oximes or carbonyl compounds amenable to oximation has not been reported. This paper describes the development of a spectrophotometric analysis of ketonic drugs through their oxime formation, followed by solvent extraction and reaction with electron acceptor reagents.

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Experimental

Reagents and Materials

All chemicals used were of analytical-reagent grade; solvents were of spectroscopic grade.

Stock standard solutions of the drugs were prepared by accurately weighing 100 mg of the cited drugs into 100-ml calibrated flasks, dissolving in absolute ethanol and diluting to volume with the same solvent. Working standard solutions of the drugs were prepared by diluting the stock standard solutions with ethanol to give solutions of 0.1 mg ml^{-1} concentration.

Hydroxylammonium chloride solution, 5% m/V in 60% ethanol.

σ-Acceptor, 2×10^{-4} M iodine solution in chloroform.

π-Acceptor, 2×10^{-4} M chloranil solution in chloroform.

Drugs. The drugs determined were progesterone, testosterone propionate and nandrolone phenylpropionate powders, griseofulvin powder, carvone and menthone (Aldrich), oil of caraway and oil of peppermint.

Instrument

A double-beam spectrophotometer (Uvidec-320) with 1-cm quartz or glass cells was used.

Procedures

Oximation step

To an ethanolic solution equivalent to 10 mg of the drug were added 4 ml of hydroxylammonium chloride reagent and a drop of glacial acetic acid, and the mixture was refluxed on a water-bath for 1 h (Δ^4-3-ketosteroids), or 20 min (other ketones). The reaction mixture was then evaporated to dryness at 70 °C and the residue was transferred into a 100-ml separating funnel with the aid of 10 ml of each of chloroform and water. The mixture was then shaken and the chloroform layer separated and transferred into a 100-ml calibrated flask. Extraction was continued with six successive 10-ml portions of chloroform, which were collected in the same calibrated flask. The extract was diluted to volume with chloroform and then subjected to either Procedure A or Procedure B.

Colour development

Procedure A. A 0.5–1-ml aliquot of the ketoxime extract was transferred into a 10-ml calibrated flask, treated with 2 ml

of iodine solution and allowed to stand for 30 min, before diluting to volume with chloroform. The absorbance of the solution was measured at 300 nm (ketosteroids) or 295 nm (griseofulvin, carvone and menthone) in a 1-cm cell against a reagent blank.

Procedure B. A 1-ml aliquot of the extract was transferred into a 10-ml calibrated flask, treated with 5 ml of chloranil solution and allowed to stand for 45 min at room temperature, or placed in a water-bath at 45 °C for 15 min, cooled and then diluted to volume with chloroform. The absorbance of the solution was measured at 430 nm (ketosteroids) or 440 nm (other ketones) in a 1-cm cell against a reagent blank.

The concentration of the drug was calculated from the calibration graph obtained by applying either procedure A or B to standard solutions equivalent to 1–5 mg of drug per 100 ml, or from the corresponding linear equation describing the calibration graph.

Application to Δ^4-3-ketosteroid drugs in ampoules

An accurately measured volume of the injection equivalent to 10 mg of the drugs was dissolved in 40 ml of light petroleum (40–60 °C, saturated with 90% ethanol) and extracted with four 20-ml portions of 90% ethanol (saturated with light petroleum). The alcoholic extracts were collected in a 100-ml calibrated flask and diluted to volume with 90% ethanol. A portion was subjected to the oximation and colour development procedures described above.

Application to griseofulvin tablets

Twenty tablets were finely powdered and an accurately weighed amount equivalent to 40 mg of drug was refluxed with 75 ml of absolute ethanol for 15 min. Sufficient ethanol was added to dilute to 100 ml, the mixture was centrifuged and a 25-ml aliquot of the supernatant liquid was subjected to the oximation and colour development procedures.

Application to caraway or peppermint oil

A 1-ml volume of the volatile oil was diluted to 100 ml with ethanol and then 1 ml of the resulting solution was subjected to the oximation procedure, followed by procedure A or B.

For the standard additions method, a suitable aliquot of the standard solution of carvone or menthone was added to a 1-ml aliquot of the previously analysed diluted oil, then the assay was carried out and the recovery of the added amount was calculated.

Results and Discussion

Oximation

The addition - elimination oximation reaction of the studied compounds is assumed to give an oxime according to the following route:

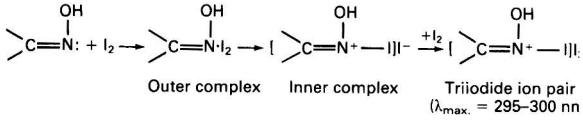
The authors' earlier studies[7,15] of the optimum conditions for carrying out this reaction have led to the described conditions of oxime preparation and manipulation of the reaction mixture prior to its solvent extraction. Chloroform, the chosen extraction solvent, was also an appropriate medium for the charge-transfer complexation reaction, as it has the desired solubilising and lipotropic characteristics, in addition to a reasonable degree of polarity.

Stoicheiometry

As carvone, menthone, testosterone propionate and nandrolone phenylpropionate contain only one carbonyl group, they should give a monoxime on reaction. Although progesterone and griseofulvin contain two keto groups per molecule, they also gave a monoxime, a fact which was noted in the literature[15,31] and in this work from molar absorptivity calculations applying Job's[33] method and other experimental observations.

Iodine Acceptor Procedure

Oximation results in the introduction of a basic centre to the carbonyl drug molecule. Mixing the oxime and iodine in chloroform media resulted in a change of the violet colour of iodine to yellow, owing to a charge-transfer complexation reaction between the ketoxime n-donor and the σ-electron acceptor iodine followed by the formation of a triiodide ion pair, as shown by the following suggested routes, which agree with reports on similar reactions in the literature[25,26]:



The absorption graphs of the reaction products (Figs. 1 and 2) show a high absorption band in the region of 300 nm and a lower band with a maximum at 365 nm, characteristic of the n-donor - iodine charge-transfer complexes. In chloroform, iodine itself has a maximum absorption at about 512 nm and the free oxime has an absorption peak at 340–350 nm. However, the iodine - oxime product has an absorption maximum at 270–300 nm. This variation is due to the modification of the absorption maximum of the triiodide ion formed from the release of iodide ions in the reaction by the accompanying ketoxime cation.[25,26,32]

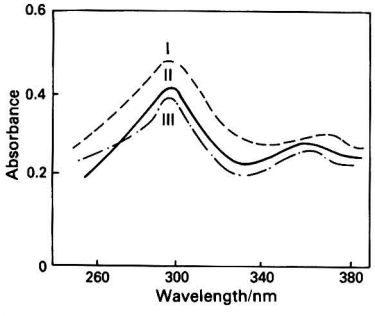


Fig. 1. Absorption spectra of oxime - iodine complexes in chloroform for: I, griseofulvin (0.019 mg ml^{-1}); II, carvone (0.016 mg ml^{-1}); and III, menthone (0.02 mg ml^{-1})

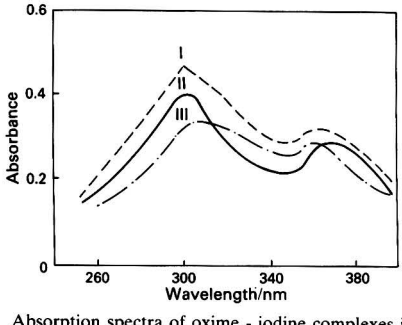
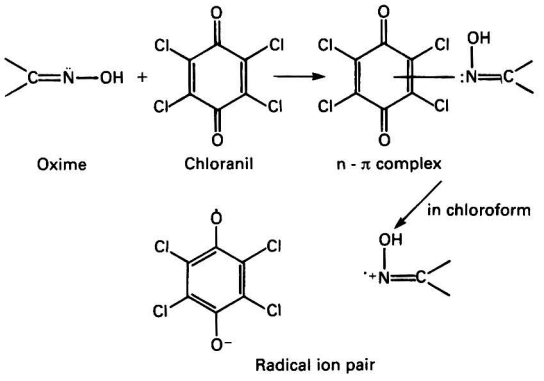


Fig. 2. Absorption spectra of oxime - iodine complexes in chloroform for: I, progesterone (0.025 mg ml^{-1}); II, testosterone propionate (0.028 mg ml^{-1}); and III, nandrolone phenylpropionate (0.03 mg ml^{-1})

Chloranil Acceptor Procedure

Mixing the chloroform oxime extract ($\lambda_{max.}$ 340–350 nm) with chloroformic chloranil solution ($\lambda_{max.}$ 290 nm) resulted in the development of a red chromogen. The colour reached its maximum intensity and stability on standing for 45 min at room temperature or on heating in a water-bath at 45 °C for 15 min. This interaction is due to charge-transfer complexation between the ketoxime, n-donor and the chloranil π-acceptor, which, because of the polarity of the medium, may lead to a radical ion pair[28] as shown in Scheme 1. The chloranil radical ion pair had a $\lambda_{max.}$ of 430–440 nm (Figs. 3 and 4). The $\lambda_{max.}$ of the chloranil radical ion would hence be slightly modified by the accompanying ketoxime cation, otherwise different ketoxime complexes would have given an identical $\lambda_{max.}$



Scheme 1

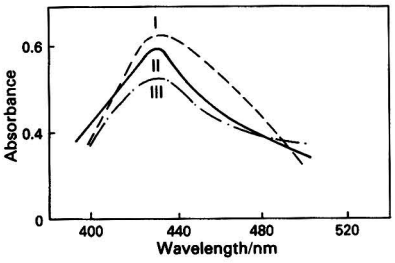


Fig. 3. Absorption spectra of oxime - chloranil complexes in chloroform for: I, progesterone (0.045 mg ml^{-1}); II, testosterone propionate (0.045 mg ml^{-1}); and III, nandrolone phenylpropionate (0.044 mg ml^{-1})

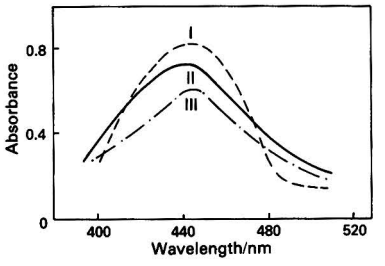


Fig. 4. Absorption spectra of oxime - chloranil complexes in chloroform for: I, griseofulvin (0.033 mg ml^{-1}); II, carvone (0.038 mg ml^{-1}); and III, menthone (0.035 mg ml^{-1})

Table 1. Beer's plot data for ketoxime charge-transfer complexes in chloroform

	Iodine acceptor (procedure A)		Chloranil acceptor (procedure B)	
Drug	Slope (b)	Intercept (a)	Slope (b)	Intercept (a)
Progesterone	0.174	0.04	0.200	−0.02
Testosterone propionate	0.145	0	0.181	−0.01
Nandrolone phenylpropionate	0.122	−0.01	0.154	−0.03
Griseofulvin	0.242	0.02	0.262	−0.03
Carvone	0.215	0.02	0.200	0
Menthone	0.198	−0.01	0.162	0.02

Table 2. Results of assay of pharmaceutical carbonyl compounds using iodine acceptor charge-transfer complexation method (procedure A) and the BP 1980 method

	Recovery* ± S.D., %			
Preparation	Proposed method	BP method	*t* (2.3)†	*F* (6.39)†
Pure progesterone	100.15 ± 0.38	100.19 ± 0.73	0.11	3.65
Progesterone (Lutone ampoules)	99.99 ± 0.41	99.63 ± 0.44	1.38	1.12
Pure testosterone propionate	100.31 ± 0.20	100.47 ± 0.51	0.66	6.30
Testosterone propionate (Testone E. ampoules)	99.97 ± 0.39	99.71 ± 0.77	0.66	4.0
Pure nandrolone phenylpropionate	100.14 ± 0.53	99.96 ± 0.49	0.54	1.12
Nandrolone phenylpropionate (Durabolin ampoules)	99.84 ± 0.35	100.02 ± 0.63	0.54	3.25
Pure griseofulvin	99.87 ± 0.55	100.11 ± 0.7	0.6	1.63
Griseofulvin (Griseofulvin tablets)	99.98 ± 0.74	100.26 ± 0.66	0.62	1.25
Pure carvone	100.19 ± 0.65	100.52 ± 0.88	0.69	1.83
Carvone (in caraway oil)	99.75 ± 0.45	98.98 ± 0.55	2.28	1.5
Pure menthone	100.21 ± 0.62	100.41 ± 0.68	0.49	1.2
Menthone (in peppermint oil)	99.75 ± 0.45	99.55 ± 0.52	0.66	1.35

* Mean of five determinations ± standard deviation.
† Figures in parentheses are tabulated values of *t* and *F* at the 95% confidence limit.

Table 3. Results of assay of pharmaceutical carbonyl compounds using chloranil acceptor charge-transfer complexation method (procedure B) and the BP 1980 method

Preparation	Recovery* ± S.D., %		*t* (2.3)†	*F* (6.39)†
	Proposed method	BP method		
Pure progesterone	100.20 ± 0.66	100.19 ± 0.73	0.02	1.20
Progesterone (Lutone ampoules)	100.27 ± 0.47	99.63 ± 0.44	2.28	1.16
Pure testosterone propionate	100.42 ± 0.49	100.47 ± 0.51	0.16	1.08
Testosterone propionate (Testone E. ampoules)	99.77 ± 0.50	99.71 ± 0.77	0.15	2.40
Pure nandrolone phenylpropionate	100.01 ± 0.41	99.96 ± 0.49	0.18	1.47
Nandrolone phenylpropionate (Durabolin ampoules)	99.76 ± 0.44	100.02 ± 0.63	0.76	1.95
Pure griseofulvin	100.10 ± 0.77	100.11 ± 0.70	0.02	1.22
Griseofulvin (Griseofulvin tablets)	100.34 ± 0.60	100.26 ± 0.66	0.20	1.22
Pure carvone	99.95 ± 0.52	100.52 ± 0.88	1.26	2.85
Carvone (in caraway oil)	99.49 ± 0.40	98.98 ± 0.55	1.70	1.87
Pure menthone	100.19 ± 0.49	100.41 ± 0.68	0.59	1.96
Menthone (in peppermint oil)	99.99 ± 0.59	99.55 ± 0.52	1.29	1.29

* Mean of five determinations ± standard deviation.
† Figures in parentheses are the tabulated values of *t* and *F* at the 95% confidence limit.

Assay Parameters

Effect of temperature

After its full development, the complex may dissociate with a corresponding decrease in intensity on heating above room temperature.

Effect of complex formation

Leaving the reaction mixture to stand, as described under Procedures, was essential for the complete development of the complexes by transformation of the outer complexes to inner complexes.[32]

Effect of varying acceptor concentration

The concentration of the acceptor should not be excessive in order to avoid the formation of ter-molecules or higher complexes.[32]

Linearity of Calibration Graphs

A linear relationship was obtained for the absorbance of the ketoxime acceptor reaction products when the concentration of the parent drugs was in the concentration range 0.01–0.05 mg ml^{-1} in the final measured solutions. The graphs show negligible or zero intercepts and are described by the regression equations $A = a + bC$ (A, absorbance of a 1-cm layer; b, slope; a, intercept; and C, concentration of the measured solution in mg per 100 ml) obtained by the least-squares method.[34] The data for the compounds studied (Table 1) that were used for the calibration graphs indicate the sensitivity of the proposed procedures.

Quantification, Accuracy and Precision of Procedures A and B

The validity of the proposed procedures for the determination of the studied compounds in their pure state and in pharmaceutical forms was tested by analysing these products with the proposed procedures and an official method.[17] A standard additions technique was used for the determination of carvone and menthone in volatile oils of caraway and peppermint, respectively, to give the percentage recovery of an added amount. The results obtained (Tables 2 and 3) were comparable to those obtained by the official method as both the *t* and *F* values did not exceed the theoretical values.[34] This indicated that the proposed procedures were as accurate and precise as the applied official methods and that no interference from excipients and vehicles was encountered. This was expected because the procedure involves solvent extraction of the oxime formed and a preliminary clean-up step with 90% ethanol was used for the injections. The procedure proved to be directly applicable to volatile oils with no preliminary separation step. The proposed procedures offer the advantages of accuracy and simplicity of reagents and apparatus.

Among the spectrophotometric methods used to determine ketones in volatile oils, the proposed procedure is one of the most sensitive, simple and accurate and is of potential use as a general method. No preliminary clean-up step before oximation is needed if the chloranil acceptor method is used to determine Δ^4-3-ketosteroid drugs in injections.

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Spectrophotometric Study of the Iron(III) – Morin Complex in a Micellar Medium

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A spectrophotometric study of the iron(III) - morin complex in the presence of several surfactants (non-ionic, cationic and anionic) at different concentrations is reported. The study was performed at pH 4, and all the surfactants used (except a non-ionic surfactant of the ethylene oxide - propylene oxide condensate group) solubilised the complex. However, maximum sensitisation could be obtained only by using non-ionic surfactants.

A subsequent spectrophotometric study in the presence of the non-ionic surfactant Nemol K-1030 (polyoxyethylenated nonylphenol) was carried out. The green - brown complex formed in micellar medium showed maximum absorption at 417 nm. Using a concentration of 8.95×10^{-6} M of Fe(III), the optimum conditions were 1.18×10^{-4} M (0.004%) morin, 1.25×10^{-2} M (1%) Nemol K-1030 and pH 3.7, adjusted with acetic acid - acetate buffer. No variations in absorbance were observed between 15 and 35 °C.

The stoicheiometry of the Fe(III) - morin complex in the presence of 1% of Nemol K-1030 was calculated by the molar ratio, continuous variations and slope ratio methods. A ratio of Fe(III) to morin of 1 : 4 was found in all instances. The mean conditional formation constant was found to be 3.90×10^{22}.

Using a morin concentration of 0.004%, the calibration graph was linear up to 1.4 p.p.m. of Fe(III), the molar absorptivity of the complex being 6.33×10^4 l mol^{-1}cm^{-1}. The detection and quantification limits were found to be 0.0038 and 0.012 p.p.m. of Fe(III), respectively. A study in the presence of different ions showed that the most important interferences are due to Zr(IV), Al(III), Ti(IV), and V(IV) and V(V).

Keywords: *Spectrophotometry; iron(III) - morin complex; surfactant*

Malat[1,2] found that the colour reaction between tin(IV) and pyrocatechol violet is greatly sensitised by adding gelatin. Since then, a number of spectrophotometric methods have been proposed in which different surfactants are used in conjunction with metallochromic reagents.[3] Thus, visible absorption methods for the determination of metal ions are improved by the use of suitable surfactants.

One improvement that has been reported from some of these procedures is due almost solely to the solubilising ability of micellar systems. Some metal ions react with an appropriate chelometric indicator or ligand to form binary metal - chelate complexes that are not soluble in water. Hence the complex must be extracted into a suitable organic solvent prior to measurement of the absorbance. However, in some instances it has been reported that the addition of a surfactant to the aqueous system renders the metal complex water soluble. This is due to the formation of an aqueous micellar system that solubilises the metal - chelate complex.[4] The metal complexes of 8-hydroxyquinoline,[5] thiazolylazonaphthol,[6] thiazolylazo-dimethylaminophenol,[7] dithizonates,[8] xylidyl blue[9] and pyridylazonaphthol[10] have been thus solubilised. Non-ionic micellar systems are typically employed in these methods,[4] although in some instances cationic surfactants can also be used as solubilising agents for water-insoluble complexes.[3]

The coloured complexes formed in micellar media are characterised by high molar absorptivities (sometimes greater than 10^5 l mol^{-1} cm^{-1}) and high stability over a wide pH range, and usually by a large bathochromic shift caused by the addition of surfactants to the binary complex formed in water.[11,12] Usually the metal - chelate complexes formed in micellar systems are much more stable than those formed in the absence of micelles.[4]

Iron has been determined spectrophotometrically using various reagents, and in the presence of different surfactants. The determination of iron with the complexing agent 2-bromo-4,5-dihydroxyazobenzene-4′-sulphonate (BDAS), using the cationic surfactant cetylpyridinium chloride (CPC) has been proposed.[13] Using the complexing agent Eriochrome Cyanine R, iron has been determined in the presence of the cationic surfactants cetyltrimethylammonium chloride (CTAC)[14] and tridodecylethylammonium bromide,[15] and in the presence of the non-ionic surfactant polyoxyethylene sorbinate lauryl ester (POESLE).[16] Analogously, Chromazurol S has been used for the determination of iron using the cationic surfactant CTAC[17] and zephiramine.[18] In these instances, the surfactant produces a bathochromic shift in the absorption maximum and an increase in the molar absorptivity.

However, the determination of iron with morin has hardly been described. In 1966, a method for the spectrophotometric determination of iron(III) by solvent extraction of the iron - morin chelate was proposed; the complex was extracted with isoamyl alcohol and the absorbance was measured at 500 or 600 nm at pH 4. The method was linear between 0.1 and 0.7 μg ml^{-1} of Fe(III).[19] In the same year, Elbeih and Abou-Elnaga[20] proposed a photometric determination of iron (III) after paper chromatographic separation; the absorbance of the morin complex was measured in an ethanolic medium at 433 nm and at an optimum pH of 5. Beer's law was obeyed in the range 0–1.5 μg ml^{-1}. Paletskite and Finkelshteinaite[21] studied the reaction of iron with morin and quercetin, and proposed a stoicheiometry of 1 : 2 for the iron - morin and 1 : 1 for the iron - quercetin complex.

In this work, the influence of different kinds of surfactant on the absorbance of the iron - morin complex was studied. A subsequent spectrophotometric study of the iron - morin system in the presence of the non-ionic surfactant Nemol K-1030 (polyoxyethylenated nonylphenol) was made in order to find the optimum conditions for the determination of iron, and the analytical characteristics of the method. The stoicheiometry and conditional formation constant of the solubilised complex were determined by different methods.

Experimental

Apparatus

Absorption measurements and spectra were obtained with a Shimadzu UV-240 spectrophotometer with automatic recording; 1.00-cm quartz cells were used.

A Termotronic S-389 thermostat was used to control the temperature to ±0.5 °C. pH was measured using a Crison Digilab 517 pH meter (pH ± 0.001). A conventional stalagmometer was used for surface tension measurements.

Reagents

Analytical-reagent grade chemicals were used and the water used to prepare solutions was distilled and de-ionised.

Fe(III) stock solution, 1000 p.p.m. Obtained by dissolving an ampoule of J. T. Baker iron standard solution (1.000 ± 0.002 g of Fe; substrate $FeCl_3$) in 1 l of water. Working solutions were prepared freshly by appropriate dilution of the stock solution with 1% HCl.

Morin solution. Prepared by dissolving 0.1 g of the reagent (Merck) in 100 ml of absolute ethanol.

Nemol K-1030 solution, 10% . Prepared by dissolving 10 g of surfactant (polyoxyethylenated nonylphenol; hydrophobic - lipophilic balance, HLB = 14.1; Massó y Carol)[22] in 100 ml of water.

Buffer solution, pH 3.7. Prepared by mixing 100 ml of acetic acid (Panreac) with 16.406 g of anhydrous sodium acetate (Merck) and diluting to 1 l with water.

Procedure

An aliquot of the Fe(III) standard solution (containing up to 70 μg of metal) was transferred into a 50-ml beaker and 5 ml of Nemol K-1030 solution were added. The pH was adjusted to 3.7 by adding 5 ml of acetic acid - acetate buffer solution, then 2 ml of morin solution were added and the solution was diluted with water to 50 ml. It was mixed well and the absorbance was measured after 15 min at 417 nm at 25 °C. Under these conditions the absorbance was stable for at least 24 h. For each measurement a parallel reagent blank without Fe(III) was run.

Results and Discussion

The absorbance of the Fe(III) - morin complex in the presence of several surfactants at different concentrations (between 0.04 and 5%) was measured. The non-ionic surfactants used were Nemol K-1030 (polyoxyethylenated nonylphenol; Massó y Carol), Triton X-100 (polyoxyethylene *p-tert*-octylphenol; Panreac), Genapol PF-20 (ethylene oxide - propylene oxide condensate; Hoechst) and Genapol SE-070 (polyoxyethylenated fatty alcohol; Hoechst). The anionic surfactant Humectante OZB (linear alkylbenzenesulphonate; Ciba-Geigy) and the cationic surfactants CPB (cetylpyridinium bromide; Sigma), CTAB (cetyltrimethylammonium bromide; Merck) and zephiramine (tetradecyldimethylbenzylammonium chloride; Pharmaceuticals Inc.) were also used.

Table 1. Influence of different surfactants on the Fe(III) - morin complex. Fe, 0.5 p.p.m.; surfactant, 0.1% *m/V*; pH, 4; morin, 0.004%.

Surfactant	$\lambda_{max.}$/nm	Absorbance
Non-ionic:		
Nemol K-1030	417	0.549
Triton X-100	417	0.535
Genapol SE-070	417	0.560
Genapol PF-20	—	—
Anionic:		
Humectante OZB	415	0.228
Cationic:		
CPB	429	0.180
CTAB	428	0.170
Zephiramine	429	0.180

The study of the influence of surfactants was made at the recommended[19] pH of 4. At this pH, the brownish black iron(III) - morin complex was insoluble in aqueous media. At concentrations ≥ 0.1%, all surfactants used solubilised the complex, except the non-ionic surfactant Genapol PF-20, with which the Fe(III) - morin complex precipitated at all concentrations tested. The absorbance of the solubilised complex was stable for at least for 2 h.

Table 1 gives the absorbance values measured at the absorption maximum of the binary complex, using a surfactant concentration of 0.1%. It can be seen that maximum absorbances were obtained in the presence of non-ionic surfactants, the absorption maximum being 417 nm. The anionic surfactant Humectante OZB also solubilised the complex, the absorption maximum being 415 nm. The cationic surfactants tested, in addition to solubilising the complex, produced a bathochromic shift of 11–12 nm (from 417 nm to 428–429 nm) in the absorption maximum.

The non-ionic surfactant Nemol K-1030 was used in subsequent experiments in order to find the optimum conditions and analytical characteristics of the system.

Effect of pH

The influence of pH was studied over the range 1–10, adjusted by means of HCl and NaOH solutions. Fig. 1 shows the relationship between absorbance and pH; it can be seen that the maximum absorbance is obtained at a pH between 2 and 4.5, the maximum absorption appearing at 417 nm. At pHs > 4.5, a decrease in absorbance was observed, accompanied by a bathochromic shift, whereas at pH 9 the absorption maximum occurred at 443 nm. On the other hand, the absorbance values were stable with time except at pH < 2, where the absorbances became unstable with time. Therefore, the optimum pH range was 2–4.5, where the absorbance was maximum and stable.

In the optimum pH range a study with two different buffer solutions was made: a 1 M HCl - 2 M potassium hydrogen phthalate buffer solution was chosen to adjust the pH between 2 and 4 and a 2 M acetic acid - 2 M sodium acetate buffer solution was used to adjust the pH in the range 3.4–4.5. The range of minimum error corresponded to the interval between pH 2.5 and 4, with maximum variations in the absorbance of about 5%. No differences in absorbance were observed when using the HCl - phthalate or acetic acid - acetate solution, so either buffer can be used to adjust the pH over the optimum range.

A pH of 3.7, adjusted with acetic acid - acetate buffer solution as indicated in the general procedure, was chosen as the optimum value.

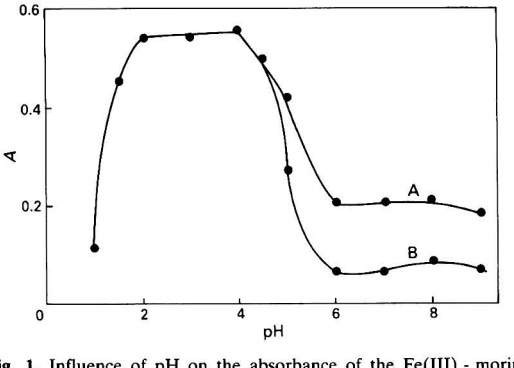

Fig. 1. Influence of pH on the absorbance of the Fe(III) - morin system in the presence of 0.1% of Nemol K-1030. Fe, 0.5 p.p.m.; morin, 0.004%. A, λ = maximum absorption for each pH (pH ≤ 4.5, 417 nm; pH 5, 429 nm; pH 6–8, 435; pH 9, 443 nm). B, λ = 417 nm

Effect of Ethanol Concentration

The influence of the concentration of ethanol on the absorbance of the Fe(III) - morin system in the presence of 0.1% Nemol K-1030 was studied. The maximum absorbance was stable up to ethanol concentrations of 10%; at higher ethanol concentrations the absorbance of the system gradually decreased, probably because ethanol destroys the micelles.

A concentration of 4% was chosen in subsequent experiments. This concentration was obtained by adding 2 ml of 0.1% morin solution in absolute ethanol, according to the general procedure.

Effect of Morin Concentration

The effect of the concentration of morin at fixed concentrations of Fe(III) (0.5 p.p.m.), ethanol (8%) and Nemol K-1030 (0.1%) was studied. The results obtained are shown in Fig. 2. It can be seen that the absorbance was maximum and stable for morin concentrations in the range 0.001–0.005%.

This study at different concentrations of morin was repeated using a higher concentration of surfactant, in order to avoid the decrease in absorbance observed at high concentrations of morin. Fixed concentrations of Fe (0.5 p.p.m.), ethanol (8%) and surfactant (1%) were used and the pH was adjusted to 3.7 with acetic acid - acetate buffer. Fig. 2 shows that when the surfactant concentration was increased the absorbance was constant up to 0.02% of morin. At higher morin concentrations a small decrease in absorbance was observed.

Effect of Surfactant Concentration

The effect of the concentration of Nemol K-1030 on the absorbance of the Fe(III) - morin system was studied, maintaining fixed concentrations of Fe (0.5 p.p.m.) and morin (0.004%) and a pH of 3.7.

Solubilisation of the Fe(III) - morin complex was observed only at surfactant concentrations higher than 0.03%, which is higher than the critical micellar concentration (CMC) of Nemol K-1030, calculated as indicated below. Thus, below the surfactant CMC, precipitation or turbidity was observed, which is in accordance with other workers.[4,23] At concentrations between 0.03 and 0.1%, the absorbance gradually increased with increasing surfactant concentration. For concentrations higher than 0.1% the absorbance was maximum and stable over a wide range of surfactant concentrations (between 0.1 and 5%).

A concentration of 1% *m/V* (1.25×10^{-2} M) was chosen for subsequent experiments.

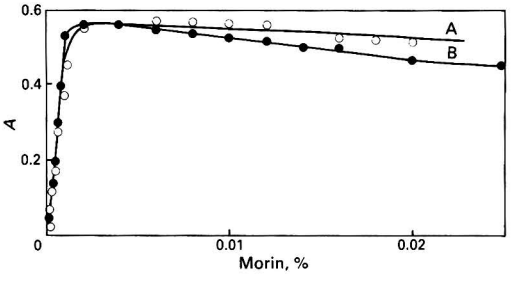

Fig. 2. Influence of morin concentration on the absorbance of Fe(III) - morin system in the presence of Nemol K-1030. Fe, 0.05 p.p.m.; pH, 3.7; λ, 417 nm. A, 1% Nemol K-1030; B, 0.1% Nemol K-1030

Table 2. Stoicheiometry and conditional formation constant of the Fe(III) - morin complex in a micellar medium (1% Nemol K-1030). pH, 3.7; λ, 417 nm

Method	M : L	Conditional constant
Molar ratio:		
c_M constant*	1 : 4.08	1.00×10^{23}
c_L constant†	1 : 3.94	1.20×10^{22}
Continuous variations‡	1 : 4.00	5.16×10^{21}
Slope ratio	1 : 3.92	—

* $c_M = 8.95 \times 10^{-6}$ M.
† $c_L = 5.91 \times 10^{-5}$ M.
‡ Total concentration = 5.37×10^{-5} M.

Determination of CMC

In order to establish whether the solubilisation of the complex and the enhanced absorbance are due to a micellar phenomenon, it is necessary to know the surfactant concentration at which the micelles are formed (CMC).

The CMC of Nemol K-1030 in water, buffered at pH 3.7 with acetic acid - acetate buffer, was 1.04×10^{-4} M (0.008%), determined by surface tension measurements. The presence of 1.18×10^{-4} M morin slightly decreased the CMC to 0.75×10^{-4} M (0.006%).

The decrease in the CMC in the presence of dyes seems to be caused by the formation of mixed micelles of dye ions and surfactant at well below the CMC.[24] In these instances, it is said that the dye induces micelle formation.

Effect of Temperature

The effect of temperature on the absorbance of the Fe(III) - morin complex in the presence of 1% Nemol K-1030 was studied. The study was performed at temperatures between 15 and 50 °C. No differences in the maximum absorption were observed when the temperature was varied between 15 and 35 °C. However, below 25 °C the development of the colour was slower and it was necessary to wait at least 30 min before measuring the absorbance. Above 25 °C, maximum absorbance was obtained in 5 min. Moreover, at temperatures higher than 35 °C the absorbance gradually decreased with increasing temperature until it reached 50 °C, which was the maximum temperature employed.

Determination of Stoicheiometry and Formation Constant

The composition of the Fe(III) - morin complex in the presence of 1% Nemol K-1030 was calculated by the molar ratio, continuous variations and slope ratio methods (Fig. 3). A molar ratio of Fe(III) to morin of 1 : 4 was found by all methods. The values obtained for the stoicheiometry and conditional formation constant are given in Table 2. A mean value of 3.90×10^{22} for the conditional constant was obtained.

The stoicheiometry of 1 : 4 does not coincide with the 1 : 2 proposed by Paletskite and Finkelshteinaite[21] for the Fe(III) - morin complex in the absence of surfactant. This difference can be explained by the micellar system producing a novel reaction medium in which the stoicheiometry can be affected.[4] This is in concordance with the work of other authors, indicating that the micellar medium seems to favour elimination of water (or hydroxide ligands) from the chelate giving rise to an increase in the coordination number of the cation.[25]

Analytical Characteristics

Linearity range

The calibration graph showed that the complex obeys Beer's law up to 1.4 μg ml^{-1} of Fe(III). The apparent molar absorptivity was calculated to be 6.33×10^4 l mol^{-1} cm^{-1} at 417 nm.

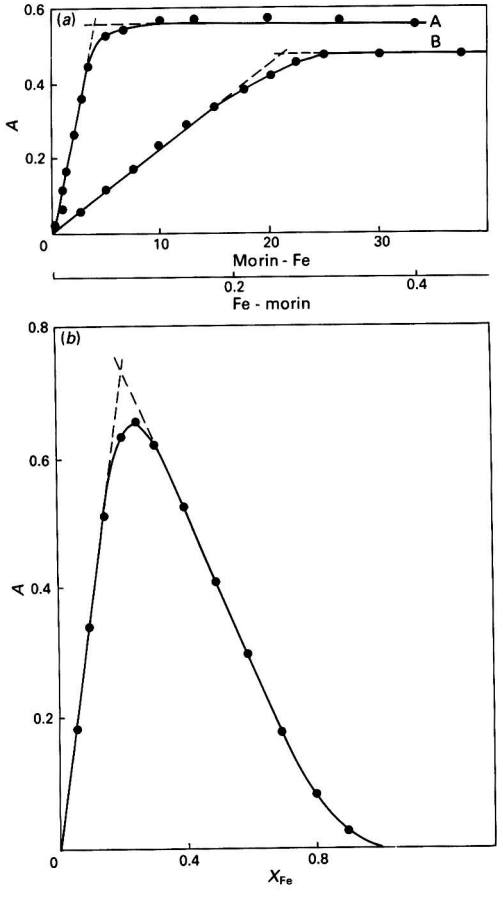

Fig. 3. Determination of stoicheiometry and conditional formation constant of the Fe(III) - morin complex in micellar medium (Nemol K-1030). Nemol K-1030, 1%; pH, 3.7; λ, 417 nm. (*a*) Molar ratio method. A, c_{Fe} constant = 8.95×10^{-6} M; B, c_{morin} constant = 1.18×10^{-4} M. (*b*) Continuous variations method for Fe to morin ratio. Total concentration, 5.37×10^{-5} M

A Ringbom plot showed that the zone of minimum spectrophotometric error corresponded to the interval 0.2–0.8 p.p.m. of Fe(III).

Precision

The relative standard deviation (s_r), evaluated from eleven independent determinations of 0.02 p.p.m. of Fe(III), was 5.9%. When the concentration of metal was increased to 0.2, 0.6 and 1.2 p.p.m., s_r was 0.54, 0.24% and 0.18%, respectively.

Detection and quantification limits

The detection limit, obtained from the sensitivity of the calibration graph and for $3s_b$ [s_b = standard deviation of a blank without Fe(III), n = 11], was found to be 0.0038 p.p.m. of Fe(III). The quantification limit, obtained for $10s_b$, was 0.0128 p.p.m. of Fe(III).

Interferences

The effect of 30 ions on the absorbance of a solution containing 0.2 p.p.m. of Fe(III) was studied. The tolerance in the measurements of absorbance was taken to be twice the relative standard deviation of the method (1.08%), *i.e.*, an interference is tolerated if its effect on the absorbance signal is less than twice the relative standard deviation.

Table 3. Tolerance to foreign ions. Fe, 0.2 p.p.m.; morin, 0.004%; Nemol K-1030, 1%; pH, 3.7; λ, 417 nm

Ion	Limiting concentration, p.p.m.	Ratio, c_{ion}/c_{Fe}
Cl^-	>100	>500
HCO_3^-	>100	>500
F^-	20	100
NO_3^-	>100	>500
HPO_4^{2-}	>100	>500
SO_4^{2-}	>100	>500
SiO_3^{2-}	20	100
Al(III)	0.005	0.025
Ba(II)	>100	>500
Be(II)	1	5
Ca(II)	>100	>500
Cd(II)	>100	>500
Co(II)	10	50
Cr(III)	20	100
Cr(VI)	0.5	2.5
Cu(II)	0.2	1
Hg(II)	>100	>500
K(I)	>100	>500
Mg(II)	20	100
Mn(II)	>100	>500
Mn(VII)	2	10
Na(I)	>100	>500
Ni(II)	10	50
Pb(II)	1	5
Sn(II)	2	10
Ti(IV)	0.02	0.1
V(IV)	0.1	0.5
V(V)	0.1	0.5
Zn(II)	5	25
Zr(IV)	0.006	0.03

Table 3 shows the limiting concentrations and ratios to Fe(III) tolerated for the different ions studied. It can be considered that none of the anions studied produce interference. The high tolerance for F^- (20 p.p.m.), which generally interferes in other methods for the determination of Fe(III), is emphasised. Al(III), Zr(IV) and Ti(IV) produce strong interferences and must be absent if Fe(III) is to be determined; their complexation with morin has been extensively described.[26] V(IV), V(V), Cu(II) and Cr(VI) can be tolerated at concentrations up to 0.1, 0.2 and 0.5 p.p.m. Be(II) and Pb(II) can be present in an excess of up to five times the Fe(III) in the sample.

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Spectrophotometric and High-performance Liquid Chromatographic Determination of the Kinetics and Mechanisms of Hydrolysis, Isomerisation and Cyclisation of Both *E* and *Z* Isomers of 2-{[(2-Amino-5-chlorophenyl)phenylmethylene]amino}acetamide

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The behaviour of 2-{[(2-amino-5-chlorophenyl)phenylmethylene]amino}acetamide in aqueous solution was investigated in the pH range 1.0–8.0, with respect to the *E* or *Z* configuration around the imine bond. The *E* isomer yields kinetic data that obey the kinetic law for successive first-order reactions. The first step consists of the isometric transformation of the *E* to the *Z* isomer. The second step implies a parallel reaction mechanism, *i.e.*, either a hydrolysis yielding 2-amino-5-chlorobenzophenone or an intramolecular ring closure reaction yielding desmethyldiazepam. This parallel route constitutes a major reaction at pH values below the pK_a of the immonium - imine equilibrium, as demonstrated by UV spectrophotometry and high performance liquid chromatography.

Keywords: *2-{[(2-Amino-5-chlorophenyl)phenylmethylene]amino}acetamide;* E - Z *isomerism; hydrolysis and cyclisation; high-performance liquid chromatography; spectrophotometry*

The important role of the inhibitory neurotransmitter glycine in neuropsychiatric disorders is gaining increasing support as a result of the experimental evidence accumulated over the last few years.[1] One possible approach to the correction of dysfunctions due to a deficiency of this neurotransmitter could be to increase the glycine concentration in the central nervous system (CNS).

This line of thinking has already been applied to another neurotransmitter, GABA, and has led to the development of the anti-epileptic drug progabide.[2,3] The over-all lipophilicity of drugs in which glycinamide is reversibly attached to a very lipophilic benzophenone is sufficient to enable these molecules to pass the blood - brain barrier. Certain substitutions on the benzophenone moiety had to be envisaged, such as the introduction of a functional group at the 2 position in order to improve the stability of the imine functional group.[4]

Kinetic studies concerning the hydrolysis of 2-methoxy and 2-hydroxy derivatives have been reported.[5–8] The previous investigations indicate that: (1) for the 2-methoxy compound, the hydrolysis rate follows a kinetic law of the type $v = k_{exp}$[substrate], with $k_{exp} = k_{H+}[H_2O][H^+]$, where k_{exp} is the experimental rate constant and k_{H+} is the H^+-catalysed reaction rate constant; (2) for the various substituted 2-hydroxy compounds, the kinetic law is of the above type in acidic media whereas in neutral solutions the predominant reaction is the addition of a water molecule to the quinonoid tautomer; (3) in basic media (pH ≥ 10), the hydrolysis of 2-methoxy derivatives is uncatalysed and proceeds very slowly, and the 2-hydroxy derivatives are rapidly hydrolysed according to a kinetic law of the type $v = k_{exp}$[substrate], with $k_{exp} = k_{OH^-}[OH^-]$, where k_{OH^-} is the OH^--catalysed reaction rate constant.

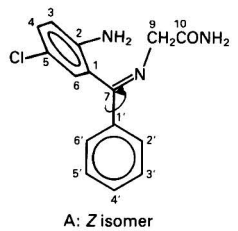
Regardless of the acidity of the medium, the rate of hydrolysis is controlled essentially by steric factors.[8] The polarity of the imine bond also needs to be considered.[7]

This work deals with the 2-amino derivatives A and B (Scheme 1). The hydrolysis rate of the azomethine bond yielding compound C was investigated in relation to the *E* or *Z* configuration around the imine bond and the conformation of the molecules. In addition, in acidic media, the *Z* isomer A is involved in an intramolecular ring-closure reaction yielding desmethyldiazepam, D.

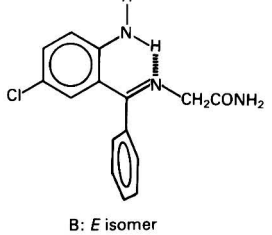
Experimental

Materials

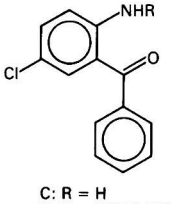
General procedures for the preparation of the benzylidene derivatives A and B have been previously reported.[4] The treatment of 2-amino-5-chlorobenzophenone, C, with ethanolamine at 135 °C gave 2-{[(2-amino-5-chlorophenyl)phenylmethylene]amino}ethanol, which was dissolved in a solution of glycinamide hydrochloride in methanol, heated at 60 °C for 6 h and then poured into water. The product was extracted with chloroform and the extracts were washed with water, dried and evaporated. The NMR spectrum showed that this residue was a mixture of *Z* (A) and *E* (B) isomers (65 + 35).



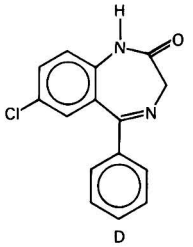
A: *Z* isomer



B: *E* isomer



C: R = H
E: R = $COCH_2NH_2$



D

Scheme 1

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The residue was washed with diethyl ether; the ether-insoluble part gave A (48%) by recrystallisation from methanol, and the ether solution evaporated to dryness and recrystallised from ethanol gave B (28%).

(Z)-2-{[(2-*Amino*-5-*chlorophenyl*)*phenylmethylene*]*amino*} *acetamide* (*A*). M.p. 180 °C (decomp.). Found: C, 62,55; H, 4.88; N, 14.48; Cl, 12.45%. $C_{15}H_{14}ClN_3O$ requires C, 62.61; H, 4.90; N, 14.60; Cl, 12.32%. $\gamma_{max.}$ ($CHCl_3$) 3510 and 3390 (NH_2), 1690 (C=O) and 1625 cm^{-1} (C=N); $\lambda_{max.}$ (MeOH) 300 (shoulder), 248 and 206 nm (ε_{300} 2.25 × 10^3, ε_{248} 2.40 × 10^4 and ε_{206} 3.86 × 10^4); δ_H (200 MHz; $CDCl_3$) 3.56 (2H, br s, 2-NH_2), 3.96 (1H, d, *J* 18 Hz, 9-H), 4.07 (1H, d, *J* 18 Hz, 9-H), 5.82 and 7.30 (1H, br s, and 1H, br s, $CONH_2$), 6.72 (1H, d, *J* 9 Hz, 3-H), 6.87 (1H, d, *J* 2.5 Hz, 6-H), 7.20 (1H, dd, *J* 9 and 2.5 Hz, 4-H), 7.52–7.35 (3H, m, 3′-H, 4′-H and 5′-H) and 7.72 p.p.m. (2H, m, 2′-H and 6′-H); δ_C (50.32 MHz; $CDCl_3$) 56.4 (C-9), 117.4 (C-3), 122.7 (C-1), 123.6 (C-5), 127.3 (C-6), 128.2 (2C; C-2′ and C-6′), 128.8 (2C; C-3′ and C-5′), 130.3 (C-4), 131.5 (C-4′), 137.3 (C-1′), 141.4 (C-2), 167.6 (C-7) and 173.5 p.p.m. (C-10).

(E)-2-{[(2-*Amino*-5-*chlorophenyl*)*phenylmethylene*]*amino*} *acetamide* (*B*). M.p. 143–144 °C. Found: C, 62.50; H, 4.98; N, 14.64; Cl, 12.33%. $C_{15}H_{14}ClN_3O$ requires C, 62.61; H, 4.90; N, 14.60; Cl, 12.32%. $\gamma_{max.}$ ($CHCl_3$) 3510 and 3390 (free NH_2 of amide), 3470 (free NH of aniline), 3270 (bonded NH of aniline), 1690 (C=O) and 1610 cm^{-1} (C=N); $\lambda_{max.}$ (MeOH) 365, 260 (shoulder), 232 and 204 nm (ε_{365} 6.15 × 10^3, ε_{260} 7 × 10^3 ε_{232} 2.88 × 10^4 and ε_{204} 2.9 × 10^4); δ_H (200 MHz; $CDCl_3$) 3.99 (2H, s, 9-H), 6.40 and 6.49 (1H, br s, and 1H, br s, $CONH_2$), 6.56 (2H, br s, 2-NH_2), 6.67 (1H, d, *J* 8.8 Hz, 3-H), 6.78 (1H, *J* 2.4 Hz, 6-H), 7.16–7.03 (3H, m, 2′-H, 6′-H and 4-H) and 7.54–7.43 p.p.m. (3H, m, 3′-H, 4′-H and 5′-H); δ_C (50.32 MHz; $CDCl_3$) 57.0 (C-9), 118.1 (C-3), 120.5 (C-1), 120.5 (C-5), 127.1 (2C; C-2′ and C-6′), 129.2 (2C; C-3′ and C-5′), 129.2 (C-4′), 131.4 (C-4), 132.7 (C-6), 135.7 (C-1′), 147.7 (C-2), 173.2 (C-10) and 174.3 p.p.m. (C-7).

Configuration and Conformation of A and B

The IR and UV spectra of A and B were of particular interest. In the IR spectrum of A, no absorption band characteristic of the bonded NH aniline group was observed in dilute solution, but B showed an absorption band at 3270 cm^{-1} that was indicative of an internal hydrogen bridge between one of the aniline hydrogens and the imine nitrogen.

The UV absorption spectra of A and B were different; B exhibited a supplementary band at λ = 365 nm (MeOH: ε_{365} 6.15 × 10^3), suggesting the existence of a tautomeric equilibrium in a neutral medium (HA ⇌ HB).[5]

The existence of the hydrogen bridge in B (Scheme 1) forces the side chain into an *E* configuration in which the imino substituent is anti to the substituted benzene ring and compound A occurs in the *Z* configuration. The NMR spectra of A and B were also consistent with the proposed configuration. Hence the difference of 3 p.p.m. in the ^1H NMR signal of the NH_2-aniline (3.56 in A compared with 6.56 p.p.m. in B) confirmed the hydrogen bonding in B and the absence of a hydrogen bridge in A. Changes in the shifts of aromatic protons and in the ^{13}C chemical shifts gave information concerning the conjugative interactions between the phenyl rings and the imine bond.

The two phenyl groups are connected to an sp^2-hybridised carbon atom, and the tendency of the phenyl ring towards co-planarity (a gain in delocalisation energy) is counteracted by steric interactions, mainly between 6-H and 2′-H (6′-H) and between 2′-H (6′-H) and the methylene group of the side-chain. If one phenyl group assumes a position in the nodal plane of the imine bond, the other will be moved further out of this plane.

This type of steric hindrance has been studied by means of molecular models, spectroscopic methods (UV, IR, NMR, ESR) and quantum mechanical calculations.[9] From these studies it follows, as confirmed by our NMR data, that in A, the most heavily *ortho*-substituted ring disposed *cis* with respect to the imine-substituted ring makes the largest angle with the nodal plane of the C=N bond.

In compound B, a conformational consequence of the existence of a hydrogen bridge and the tautomerism observed by UV is the co-planarity of the substituted ring with the imine double bond (Scheme 1). This explains the chemical shift differences of 2′-H (6′-H) in A and B at 7.72 and 7.16–7.03 p.p.m., respectively. The chemical shifts of C-4′ in A and B (131.5 and 129.2 p.p.m., respectively) reflect the twisting angle θ of this ring with the imine bond and are free from steric compression. They may be estimated by the following approximation[10]: 4.74 $\cos^2\theta = \delta^x - \delta^{90°}$ p.p.m., where δ^x is the measured 4′-carbon atom chemical shift of A and B, $\delta^{90°}$ is the 4′-carbon atom in a totally non-conjugated derivative where the imine double bond is reduced to a C–N single bond (δ^x = 127.9 p.p.m. for the dihydro derivatives of A and B), and 4.74 is an expression of the gain in p.p.m. of maximum overlap and is derived from various model compounds.[11] The dihedral angles were found to be 57° for A and 28° for B.

The 2-amino-5-chlorobenzophenone, C, was obtained commercially (Fluka) and desmethyldiazepam, D, was synthesised by the procedure of Sternbach *et al.*[12]

Equipment

^1H and ^{13}C NMR spectra were recorded on a Bruker WP 200 SY instrument with tetramethylsilane as an internal standard. Chemical shifts are reported in p.p.m. The following abbreviations are used in describing the spectra: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, br = unresolved broad signal. The IR spectra of KBr pellets were recorded using a Perkin-Elmer 399 spectrophotometer. UV - visible absorption spectral data were obtained using a Varian Superscan 3 spectrometer. A cell compartment was used between thermoplates throughout the kinetic study and the temperatures were maintained at 10 ± 0.1 or 45 ± 0.1 °C by a circulating water-bath. When UV scanning was necessary, an automatic repetitive scanning device was used. The HPLC system consisted of a Rheodyne Model 7125 injection valve, a Model 114 M solvent delivery module, a 165 variable-wavelength detector and a 450 data system/controller, all from Beckman Instruments. All the reagents were of analytical-reagent grade. The chromatographic solvents used for the separation were of HPLC grade. The mobile phase was a ternary mixture of phosphoric acid (0.05 M; pH 3.5, adjusted with ammonia solution - acetonitrile - methanol (55 + 30 + 15) and the flow-rate was 2.5 ml min^{-1}. The separation was performed at ambient temperature using a 5-μm Spherisorb ODS-2 column (200 × 4.6 mm i.d.). The detector was set at 254 nm with a sensitivity of 0.05 a.u.f.s. The concentrations of A, B, C and D were determined by an external calibration with authentic reference compounds. The pK_a determination and pH measurements were carried out using a Tacussel T.S. 70 N1 pH meter.

Stock Solutions and Buffer

Stock solutions (0.5 mM) of compounds A and B were prepared in methanol. All the reaction rate studies were performed in aqueous methanolic buffer (8 + 2) solutions. The buffer systems used were sulphuric acid, pH ≤ 2, citric acid and phosphoric acid (0.1 M), pH 2–8. The ionic strength of each was adjusted to 0.5 M with sodium chloride.

Results and Discussion

Spectral Changes and pK_a Determination

The UV spectral characteristics of A and B in water containing 20% (*V/V*) of methanol are reported in Table 1. The spectral changes *versus* pH graphs for both compounds showed two successive steps. The first change in the pH of 0–2 did not enable us to obtain accurate quantitative information about the pK_a value for the equilibrium [$H_3A^{2+} \rightleftharpoons H_2A^+ + H^+$], owing to the fast ring-closure reaction (see below). A second change was observed in the pH range 2–7 corresponding to the equilibrium [$H_2A^+ \rightleftharpoons HA + H^+$].

The main bands exhibited by A (*Z* isomer) are listed in Table 1 and shown in Fig. 1. With increasing pH, a decrease of two of the absorption bands is observed at 275 and 390 nm. The changes in the spectra show an isosbestic point at 262 nm, indicating that a simple acid - base equilibrium is shifted. The value of pK_a was determined from graphs of $\log[(A_{H_2A+} - A)/(A - A_{HA})]$ *versus* pH, at 275 nm, where A_{H_2A+} and A_{HA} are the respective absorbances of the protonated and neutral species, and A the absorbance of their mixture at a fixed pH:

$$pH = pK_a + \log[(A_{H_2A+} - A)/(A - A_{HA})] \quad . . \quad (1)$$

The pK_a was found to be 4.20 ± 0.05 at 10 °C. At this temperature, the hydrolysis and the ring-closure reactions (see below) are not fast enough to interfere with the pK_a determination.

The same approach was used for B (*E* isomer). The main bands exhibited by B are listed in Table 1 and shown in Fig. 2.

In the pH range 2–7, the band at 352 nm increases with increasing pH. The changes in the spectra show three isosbestic points at 242, 318 and 378 nm, indicating that an acid - base equilibrium is shifted. The pK_a value was determined from graphs of $\log[(A - A_{H_2A+})/(A_{HA} - A)]$ *versus* pH:

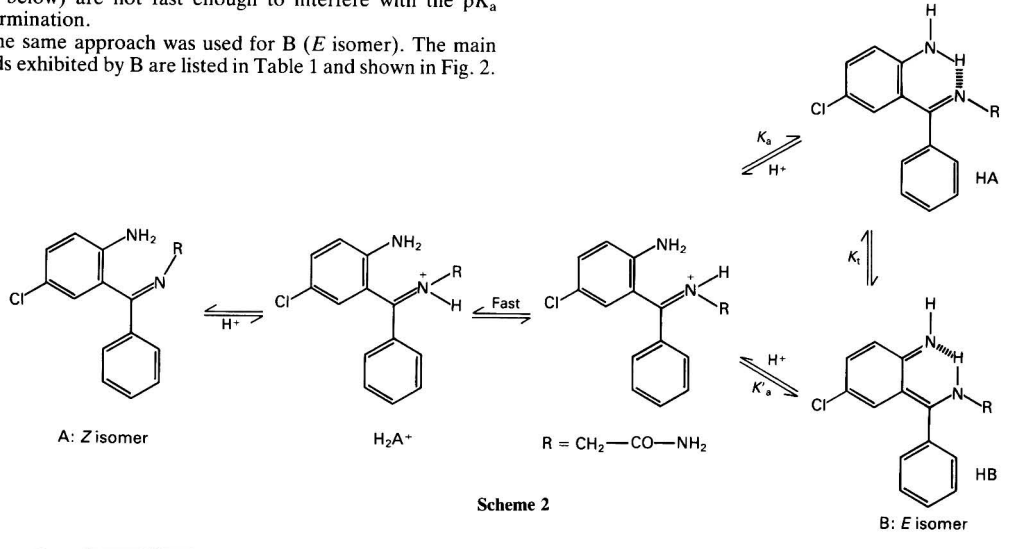
$$pH = pK_a + \log[(A - A_{H_2A+})/(A_{HA} - A)] \quad . . \quad (2)$$

The measured pK_a was apparent due to the occurrence of the tautometric equilibrium HA ⇌ HB suggested by previous work dealing with {[(2-hydroxy-5-chlorophenyl)phenylmethylene]amino}acetamide.[5] As shown in Scheme 2, the hydrogen bond initiates the tautomeric equilibrium which generates the quinonoid form HB. The over-all equilibrium is quantitatively described by the following expressions:

$$K_{app} = \frac{([HA] + [HB])[H^+]}{[H_2A^+]} = K_a + K'_a \quad . . \quad . . \quad (3)$$

$$K_t = \frac{[HB]}{[HA]} = \frac{K'_a}{K_a} \quad . . \quad . . \quad . . \quad . . \quad (4)$$

As reported previously,[13,14] a critical assumption made here was that only the quinonoid species HB may account for the



Scheme 2

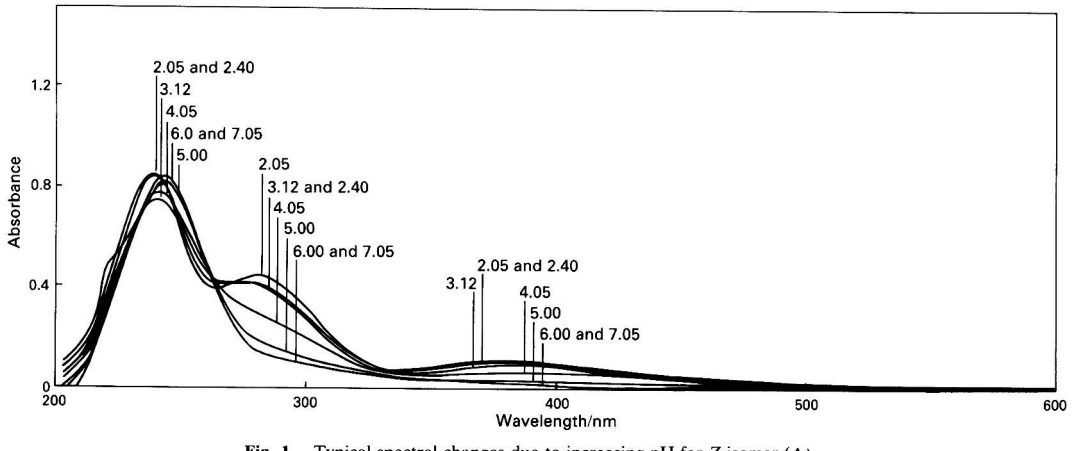


Fig. 1. Typical spectral changes due to increasing pH for *Z* isomer (A)

absorption band at 352 nm while the HA species no longer exhibits noticeable absorption at this wavelength. In agreement with this reasoning, when the azomethine bond and the *p*-chloroaniline ring are not co-planar in the *Z* isomer A, the UV - visible absorption spectrum no longer exhibits the absorption band at 352 nm (Table 1). Hence it can be deduced that this absorption band is linked to the quinonoid resonance.

In order to be able to calculate K_t, K_a and K'_a, UV - visible absorption spectra were recorded in various solvents. As shown in Table 2, the absorbance in the wavelength range 352–365 nm increases with decreasing polarity of the solvent. If it is assumed that the wavelength shift from 365 nm (in diethyl ether) to 352 nm (in water - methanol, 8 + 2) is due to the solvent effect, and if one assumes that the molar absorbances are not changed markedly by shifts from aprotic to protic solvents, then the percentage of quinonoid species HB under our experimental conditions can be deduced from the experimental ratio found for $A_{max.}$ (Table 2), *i.e.*, 4.25/6.65 = 0.64.

Hence, at 10 °C,

$$K_t = \frac{[HB]}{[HA]} = \frac{0.64}{1 - 0.64} = 1.78$$

Equations (3) and (4) enable us to determine values of K_a and K'_a. Hence, at 10 °C, $pK_a = 4.70 \pm 0.05$ and $pK'_a = 4.45 \pm 0.05$.

The pK_a values of *Z* (A) and *E* (B) isomers were found to be 4.20 and 4.70, respectively. The higher pK_a value of the *E* isomer is believed to be a consequence of the electron-donating effect exerted by the 2-amino group when the azomethine bond and the *p*-chloroaniline group are co-planar with the long-pair electrons on N-1 engaged in the quinonoid delocalisation.

The aniline nitrogen is believed to be less basic than the azomethine nitrogen for reasons similar to those for medazepam.[13,15]

The observation that acidic solutions of A or alternatively B exhibit similar UV - visible absorption spectra implies that, in acidic media, *Z* or *E* isomers give a single conjugated H_2A^+ immonium species (Table 1). Hence, it can be emphasised that the pK_a measured in the case of the *E* isomer, B, ($pK_a = 4.70$) is apparent. In this instance, the *E* to *Z* isomeric transformation is assumed to be very rapid in acidic medium, as indicated in Scheme 2.

Table 1. UV spectra of A, B, C and D [$\lambda_{max.}$, $\epsilon_{max.}$ in buffered water - methanol (8 + 2) solution]

Compound	Species	λ/nm	ε/l mol^{-1} cm^{-1} × 10^3
A, *Z* isomer ..	H_2A^+	240	21.25
		275	10.0
		390–450	2.75
	HA	243	21.0
		290(sh)	3.5
B, *E* isomer ..	H_2A^+	240	18.6
		270(sh)	10.6
		390–450	2.75
	$HA \rightleftharpoons HB$	232	27.5
		260(sh)	7.75
		352	4.25
C	H_2A^+	258	13.2
	HA	236	23.6
		258(sh)	12.0
		385	4.8
D	H_2A^+	236	30.0
		282	13.5
		360	4.0
	HA	230	35.0
		250(sh)	17.0
		310	2.0

Table 2. Ultraviolet absorption spectra of B in various solvents ($c = 5 \times 10^{-4}$ M, 25 °C)

Solvent	λ/nm	ε/l mol^{-1} cm^{-1} × 10^3
Diethyl ether	365	6.65
Dichloromethane	365	6.65
Methanol	365	6.15
Water - methanol (2 + 8) ..	360	5.50
Water - methanol (5 + 5) ..	356	5.00
Water - methanol (8 + 2) ..	352	4.25

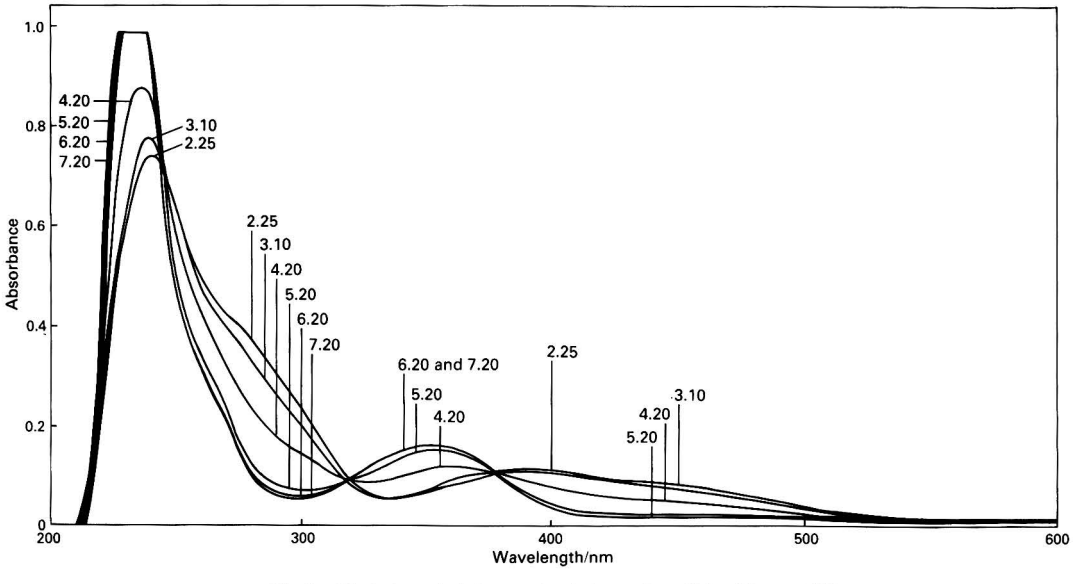


Fig. 2. Typical spectral changes due to increasing pH for *E* isomer (B)

Table 3. Experimental first-order rate constants found for isomerism (k_1), hydrolysis (k_2) and intramolecular ring closure (k_3) listed against pH

	Isomer					
	E (B)		*Z* (A)			
pH	$10^3 k_1$/min^{-1}		$10^3 k_2$/min^{-1}		$10^3 k_3$/min^{-1}	
	10 °C	45 °C	10 °C	45 °C	10 °C	45 °C
1.2					400	
1.3					320	
1.6					280	
2.0					125	
2.85			124			
3.2			120			
3.6			118			
3.85				220		
4.05			66			
4.3		190		250		
4.6			44			
5.5	7.9	29		16		6.3
6.05				9.5		3.6
6.6	1.8	7.1	0.63	3.6		1.6
7.0		4.0		1.3		1.1
7.25		3.2				

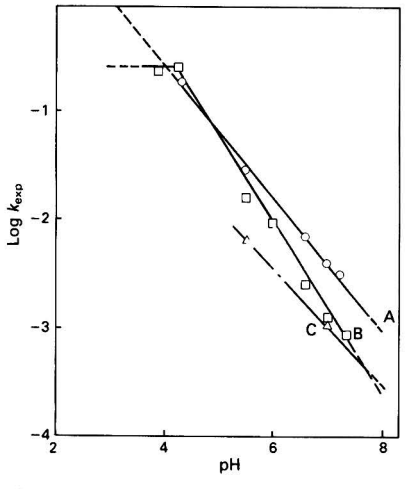


Fig. 3. Dependence of log k_{exp} on pH at 45 °C: A, isomerism transformation (k_1); B, hydrolysis (k_2); C, intramolecular ring-closure reaction (k_3)

In order to determine whether the isomeric transformation has occurred during the pK_a determination, a sample of the *E* isomer was taken at pH 2.0 and then adjusted to pH 7.0. The spectrum recorded at pH 7.0 was identical with that of a fresh sample of *Z* isomer. From this result it can be deduced that the isomeric transformation occurs rapidly in acidic medium (see Kinetic Behaviour).

Kinetic Behaviour, Rate Constant Determination and Rate - pH Profile

The reactions under consideration were the transformation of benzylidenes A and B to the benzophenone C on the one hand, and to desmethyldiazepam D on the other. The kinetic studies of the hydrolysis of A and B were followed spectrophotometrically in the pH range 1–8. The HPLC method was also used to confirm the formation of D resulting from an intramolecular ring closure.

The processes involved are presented successively for pH 4–8 and pH 1–4.

Slightly acidic and neutral media: pH range 4–8

With increasing time, the *E* isomer, B, is involved in spectral changes implying an apparent two-step reaction, both steps being first order. Spectral analysis at first showed a decrease of the band at 352 nm, followed by an increase at 385 nm. Time plots of the logarithm of absorbance, A_{352}, at any given time, and time infinity, consisted of one linear section according to the integrated first-order expression

$$\ln(A_t - A_\infty) = -k_1 t \quad \ldots \quad \ldots \quad \ldots \quad (5)$$

The apparent first-order rate constants listed in Table 3 were calculated from least-squares analysis of the slopes. The value of k_1 was found to be 29×10^{-3} min^{-1} at pH 5.5 and 45 °C. As confirmed by HPLC, the first step consists of the isomeric transformation

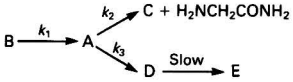
$$E \text{ isomer (B)} \xrightarrow{k_1} Z \text{ isomer (A)}$$

If the absorptivities of A, C and D at 352 nm are nearly zero, then k_1 was measured according to

$$v = \frac{dA_{352}}{dt} = -\frac{d[B]}{dt} = k_1[B] \quad \ldots \quad \ldots \quad \ldots \quad (6)$$

Moreover, the plot of log k_1 against pH is linear in the pH range 4–8 with a slope of −0.6 (Fig. 3). When extrapolated at pH values corresponding to an acidic medium, this linear section enabled us to determine the theoretical value of k_1; log k_1 was found to be 0 at pH 3.0 and 45 °C. This result implies that (*a*) the experimental determination of k is no longer possible in acidic media using the methods presented herein and (b) the conjugate acid H_2A^+ species exists predominantly as the Z isomer A in these media, as the rate constant k_1 shows high values.

The second step implies a parallel reaction mechanism, *i.e.*, either a hydrolysis of A yielding benzophenone C, or an intramolecular ring-closure reaction yielding desmethyldiazepam D, in agreement with Scheme 3. As shown in Table 1, C



Scheme 3

exhibits a well defined band at 385 nm. Moreover, in the pH range 5–8, A and D exist as free base forms HA [pK_a (A) = 4.20; pK_a (D) = 3.40], which exhibit only a negligible $A_{385\ nm}$ value and do not disturb the experimental determination of the concentration - time profile for C. Conversely, in these media, HPLC provides a convenient method for quantitative determination of [D]. From the experimental determination of [C] and [D], the value of [A] may be deduced according to the expressions

$$[A]_0 = a\,[C]_\infty + [D]_\infty$$
$$(a - x)_t = [C]_t + [D]_t$$

Using the isomer A as starting material, time graphs of the logarithm of $(a - x)$ were linear according to the integrated first-order expression

$$\ln(a - x) = -(k_2 + k_3)t \quad \ldots \quad \ldots \quad (7)$$

The value of $(k_2 + k_3)$ was found to be 22.3×10^{-3} min^{-1} at pH 5.5 and 45 °C and 2.4×10^{-3} min^{-1} at pH 7.0 and 45 °C. From the value of $[C]_\infty/[D]_\infty$ equal to 2.5 at pH 5.5 and 1.2 at pH 7.0, respectively, the individual values of k_2 and k_3 (see Table 3) may be deduced using the expression

$$\frac{k_2}{k_3} = \frac{[C]_\infty}{[D]_\infty} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (8)$$

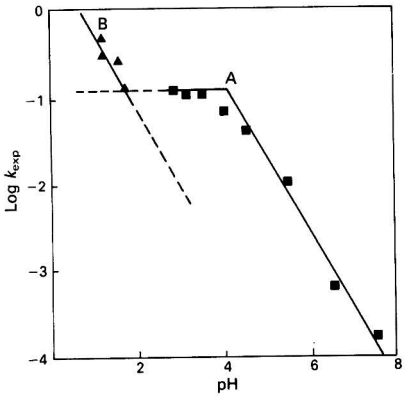


Fig. 4. Dependence of log k_{exp} on pH at 10 °C: A, hydrolysis (k_2); B, intramolecular ring-closure reaction (k_3)

Table 4. Kinetic data found for hydrolysis (k_2) and intramolecular ring closure (k_3) in the pH range 1–8

pH > pK_a (45 °C)

$$v = \frac{d[C]}{dt} = k_2[A] = k[A(HA \rightleftharpoons HB)][H_2O][H^+]^{0.8} \quad \ldots \quad (9)$$

$$v = \frac{d[D]}{dt} = k_3[A] = k'[A(HA \rightleftharpoons HB)][H^+]^{0.5} \quad \ldots \quad (10)$$

$k_2 = k_3 = 4 \times 10^{-4}$ min^{-1} at pH 7.7

pH < pK_a (10 °C)

$$v = \frac{d[C]}{dt} = k_2[A] = k[A(H_2A^+)][H_2O] \quad \ldots \quad (14)$$

$$v = \frac{d[D]}{dt} = k_3[A] = k'[A(H_2A^+)][H^+] \quad \ldots \quad (13)$$

$k_2 = k_3 = 0.126$ min^{-1} at pH 2.0

In the pH range 5–8, the log k_2 - pH relationship was a straight line (Fig. 4) whose slope (−0.8 at 10 °C and 45 °C) roughly agreed with an H$^+$-catalysed hydrolysis [expression (9), Table 4].

Using the *E* isomer (B) as starting material, from the experimental determination of the y_m/a ratio, where y_m designates the maximum concentration of the intermediate A species (Fig. 5), and a the initial concentration of starting material B, the value of the ratio k'_2/k_1 may be deduced using the mathematical procedure for the successive first-order reaction[16]

$$\frac{y_m}{a} = \left(\frac{k'_2}{k_1}\right)^{\frac{k'_2}{k'_1 - k'_2}} \quad \ldots \quad (11)$$

Using $k'_2 = k_2 + k_3 = 22.3 \times 10^{-3}$ min^{-1} at pH 5.5 and 45 °C, we found $k_1 = 30 \times 10^{-3}$ min^{-1}, which is in good agreement with the values obtained by spectral determinations at 352 nm or by HPLC, using *E* isomer (B) as starting material.

Acidic Media, pH < 4.0

In acidic media (pH ≤ 1.5), when A or B (H_2A^+ samples) were withdrawn at appropriate times and subjected to spectral analysis, the broad absorption band at 390 nm (Table 1) decreased with time, while a band appeared at 360 nm ($\varepsilon = 4 \times 10^3$ l mol^{-1} cm^{-1}). The change in spectra recorded in sulphuric acid solutions showed two isosbestic points at 320 and 410 nm indicating that a simple reaction had taken place. Time graphs of the logarithm of A_{360} at any given time, and time infinity, consisted of one linear section according to the integrated first-order expression

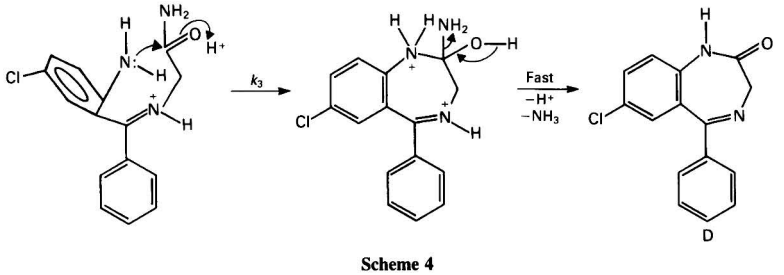
$$\ln (A_\infty - A_t) = -k_3 t \quad \ldots \quad (12)$$

By plotting the value of log k_3 at 10 °C as a function of pH, a linear section was obtained whose slope was found to be roughly equal to −1.0 (Fig. 4) in agreement with an H$^+$-catalysed phenomenon [expression (13), Table 4].

In this pH range the final spectrum recorded was identical with that of desmethyldiazepam, D. The complete formation of D was confirmed by HPLC.

These results are in agreement with the occurrence of an intramolecular ring closure H$^+$-catalysed process with NH_3 as the leaving group (Scheme 4). In less acidic media, in the pH range 1.5–3.0, the imine bond hydrolysis (k_2) and the intramolecular ring-closure reaction (k_3) constitute a competitive reaction mechanism. The UV - visible absorption spectra exhibited by C ($\varepsilon = 4.8 \times 10^3$ l mol^{-1} cm^{-1} at 385 nm) and D, H_2A^+ species, ($\varepsilon = 4.0 \times 10^3$ l mol^{-1} cm^{-1} at 365 nm) are so close to each other than the bands overlap, hence the changes in spectra did not enable us to determine the concentration - time profile for C and D. Moreover, HPLC was no longer usable for kinetic determinations owing to the high rate of the chemical reaction. However, by extrapolation of the log k_2 (10 °C) *vs.* pH and log k_3 (10 °C) *vs.* pH plots (Fig. 4) it may be deduced that at pH 2.0, $k_2/k_3 = [C]/[D] = 1.0$.

Over the range 3.0<pH<4.0, log k_2 remains independent of pH, according to expression (14) (Table 4). The intercept (Fig. 4) with the linear section in the range 4.0 < pH < 8.0 corresponds to the pK_a of the immonium imine acid - base equilibrium, in exact agreement with the value obtained from the UV - visible absorption spectra. In this pH range, the ring closure reaction would constitute a minor route with respect to



Scheme 4

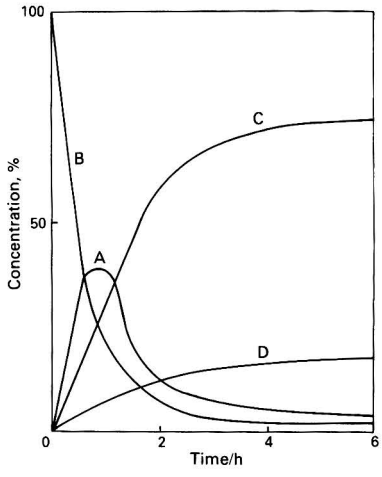


Fig. 5. HPLC concentration - time profiles for starting material B, intermediate A and products C and D; pH 5.5, 45 °C

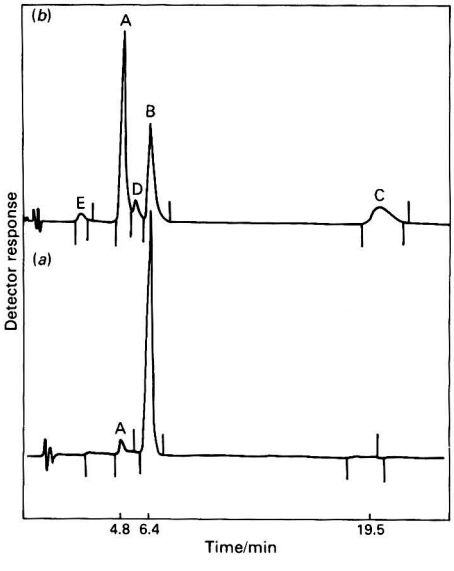


Fig. 6. HPLC profile detected at 254 nm of B hydrolysis in MeOH - water (2 + 8), pH 7.4 at 37 °C, (*a*) before initiation of the reaction and (*b*) 24 h after the reaction

the hydrolysis reaction. However, the observation that D is present in significant amounts in neutral medium [k_2 (45 °C)/k_3 (45 °C) = 1.0 at pH 7.7; Fig. 3] implies that the ring closure reaction yielding D is operable throughout the entire pH range 1–8 according to an H^+-catalysed process.

Finally, the kinetic data are consistent with the integrated first-order expressions listed in Table 4. By inspection of equations (9) and (10) and equations (13) and (14), it can be deduced that the ratio $[k_2]/[k_3]$ must decrease: (*a*) at pH > pK_a, with increasing values of pH; and (*b*) at pH < pK_a, with decreasing values of pH.

Note that product E, with the diazepine ring opened, was detected in HPLC (Fig. 6), but was not present in significant amounts under our experimental conditions, as reported previously.[17,18]

Conclusions

Compared with the behaviour of 2-{[2-hydroxy-5-chlorophenyl)phenylmethylene]amino}acetamide derivatives reported in previous papers,[4–6] the 2-amino group seems to exert a decisive influence. For 2-hydroxy derivatives in water - alcohol (8 + 2) buffered solutions, the phenol ⇌ quinonoid tautomerism equilibrium constitutes a rapid antecedent step in slightly acidic and neutral media in the over-all hydrolysis reaction step, hence yielding 2-hydroxy-5-chlorobenzophenone.

The *E* isomer (B) of 2-{[2-amino-5-chlorophenyl)phenylmethylene]amino}acetamide yields kinetic data that obey the kinetic law for successive first-order reactions. The first step consists of the isomeric transformation B to A (*Z* isomer). The second step implies a parallel reaction mechanism, *i.e.*, either a hydrolysis yielding 2-amino-5-chlorobenzophenone, C, or an intramolecular ring closure reaction yielding desmethyldiazepam, D. Only the second step occurs when the *Z* isomer, A, constitutes the starting material. The over-all process is summarised in Scheme 3.

The parallel route yielding D constitutes a major reaction at pH 2.0 and remains important in neutral media.

As far as their medical use is concerned, the 2-amino derivatives are more stable towards hydrolysis than the corresponding 2-hydroxy derivatives reported previously,[4,7] and partial cyclisation into the well known desmethyldiazepam could be of therapeutic benefit.[19]

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Sampling and Gas Chromatographic Analysis of Volatile Sulphur Compounds and Gases at Sub-v.p.m. Levels in the Presence of Ozone

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A method is presented for the sampling and analysis of sulphur gases (hydrogen sulphide and methanethiol) and volatile sulphur compounds (higher thiols and organic sulphides) in both ambient air samples and air containing ozone. The method has been developed for the quantitative analysis of hydrogen sulphide and for the semi-quantitative and qualitative analysis of other sulphur compounds. Field sampling and sample storage make use of simple cryogenic techniques and the analysis is carried out using a combination of thermal desorption and cryogenic re-trapping with sulphur-specific gas chromatography. Sample volumes of 100 ml and collection times of 1 min per sample are typically used for hydrogen sulphide in the range 0.1–1.0 volume parts per million (v.p.m.) with a limit of detection estimated to be better than 70 volume parts per billion (v.p.b.). The technique has been used in connection with odour control and measurement at a sewage treatment works, and in the assessment of odour control plant effectiveness.

Keywords: *Sulphur gas analysis; gas chromatography; thermal desorption trapping and pre-concentration; cryogenic techniques*

Sulphur gases such as hydrogen sulphide (H_2S) and methanethiol (MeSH), together with volatile sulphur compounds, for example simple thiols, and organic sulphides and polysulphides, are often implicated in nuisance odours at sewage treatment and disposal works. Levels of hydrogen sulphide in associated buildings, pump houses, wet wells, etc., can be high enough to necessitate ventilation to reduce the level of gas to a safe concentration, which may further aggravate the local odour problem. Various methods have been employed to reduce the ambient levels of sulphur compounds with varying degrees of success, for example wet or dry ozonolysis and chemical scrubbing of airborne compounds, or the dosing of sewage mains with oxidants or disinfectants such as hydrogen peroxide or sodium hypochlorite.

Frequently, the assessment of the effectiveness of control measures tends to be subjective as a result of the insensitivity of measurement techniques, and often no attempt is made to relate plant performance to known odour threshold levels.[1,2]

At a local sewage pumping station, odour complaints and high levels of hydrogen sulphide led to the present investigation being deployed to assess the optimum operating conditions for control plant using wet ozonolysis to remove the sulphur and other odorous compounds. Hydrogen sulphide was considered to be the most important odorous compound, whereas other sulphur compounds were suspected of being present intermittently.

The availability of gas chromatographic equipment and suitable specific detectors provided the basis of a method, although it was uncertain as to whether the conventional gas sampling methods were either sensitive enough or suitable for the reactive compounds involved. The distance of the site from the laboratory meant that samples would have to be transported and stored easily; the presence of ozone in the samples taken from the plant meant that losses of sulphur compounds during sampling and storage could be considerable if steps were not taken to minimise oxidation reactions. Finally, the complexity and special requirements of the gas chromatography equipment precluded the use of the instrumentation on site, which might have been the ideal solution.

A number of papers[3–9] have described methods for similar analytical requirements. Although several of the methods meet the requirements for the sensitivity and range required for sulphur compounds, most were considered unsuitable for reasons of difficulty in sampling, large sample volume requirements or excessively long sampling times, and it was felt that none were likely to succeed in the presence of ozone.

The extreme volatility of hydrogen sulphide and methanethiol (boiling-points −60.7 and 6.2 °C, respectively,[10] indicated that a cryogenic technique offered the best chance of success and would have the added advantage of slowing post-sampling reactions. Methanethiol in particular was likely to decompose if heated to temperatures much in excess of 100 °C,[3] which precluded the use of techniques involving high-temperature desorption or manipulation.

Our investigation indicated that methanethiol and its decomposition product, dimethyl disulphide, were both present at the treatment works. System inertness was an important factor in the study and the determinands did not come into contact with materials other than glass, fluorinated polymers, ceramics or silicone rubbers. A solid adsorbent, Tenax GC (2,6-diphenylphenylene oxide) was chosen as a trapping material, as it has been shown to be suitable as a column packing in the gas chromatography of sulphur compounds,[5] and liquid argon (boiling-point −186 °C) was chosen as the cryogenic medium in order to allow the use of nitrogen as a carrier gas in the chromatograph. Finally, a small portable gas sampling pump, primarily used for personal exposure monitoring, was used for field sampling to give the option of a wide range of sample volumes with the minimum of equipment.

Experimental

Equipment

Gas chromatograph

A Pye Unicam GCV with a flame photometric detector (FPD), sulphur filter and temperature programming facility was used in the investigation. The carrier gas was oxygen-free nitrogen gas and zero grade hydrogen and compressed air were used for flame gases. All the gases were purified by passing through a Type 13X molecular sieve.

Analytical column

A pre-packed glass column (2 m × 2 mm i.d.) filled with 60–80-mesh Carbopack B with a 1.5% XE-60 and 1.0% phosphoric acid stationary phase was used (Supelco).

Sample injection and flow switching valves

Inert low-pressure valves (Rheodyne Model 5020) with a 100-μl loop were used for the syringe injections.

Thermal desorber

A GN Concentrator (GN Instrumentation), fitted with PTFE transfer lines, a glass-lined stainless-steel cold-trap loop and additional flow and metering valves was used.

Computing integrator

A Hewlett-Packard 3380A or Trilab 2000 (Trivector Systems) was employed.

Gas calibration equipment

Calibration gas and pure air were mixed at a ratio of 1 : 100 using suitable flow meters (Platon Flowbits) and further diluted serially with flow meters to give an over-all dilution ratio of between 1 : 1000 and 1 : 10 000 at a final flow-rate of 5 l min^{-1}. The mixing of gases at each dilution stage took place in 300 × 30 mm Perspex tubes containing glass Raschig rings, and the final mix was sampled from a 250-ml round-bottomed flask into which the gas flowed.

Static gas standards were prepared in a 300 × 300 × 300 mm Perspex cabinet equipped with a circulating fan, a septum port for syringe or adsorbent tube sampling and a compressed air inlet for flushing.

The approximate concentration of hydrogen sulphide used for dynamic gas calibration was monitored using a hydrogen sulphide monitor (Neotronics Model HS 102) and potentiometric recorder (Servoscribe Model 1S).

Digital thermometer

A Comark Model 5000 thermometer equipped with Type K thermocouples was used.

Dewar flasks

Various sizes of vacuum flasks for handling liquid argon and sample storage (Thermos and Day-Impex) were used.

Adsorbent tubes

Pyrex glass tubes, 6 mm o.d., 4 mm i.d. and 89 mm long with their ends cut square, packed with approximately 100 mg of 60–80-mesh Tenax GC and plugged at each end with glass-wool, were used. The packing should be as central in the tube as possible, leaving the ends empty, although precise amounts and positioning of the packing is not critical.

Tubes should be conditioned for at least 1 h, six at a time, on a manifold of stainless-steel Swagelok union Tees at a temperature of 250 °C while purging with nitrogen at approximately 40 ml min^{-1} per tube.

Once conditioned, the tubes were capped with plastic caps and stored in the dark at room temperature before use. Tubes prepared in this way showed no response for any of the compounds studied after storage for periods of several weeks, although for critical applications it would be advisable to recondition prior to use.

Sampling blocks

Blocks, 60 × 60 × 60 mm, were cut from suitable polystyrene foam. A high-density foam is the most suitable. A hole was cut using a cork borer, in one face of the block, of 30 mm diameter and 45 mm deep, thus providing a container for the liquid argon. A second hole was cut completely through the block, approximately 15 mm below the top of the first hole, and at right-angles to the hole. This hole should be small enough to provide a tight fit when a sampling tube is pushed through.

The final configuration allows a sampling tube to have its central portion immersed in liquid argon with its ends protruding from the block for attachment of tubing.

Sampling pump

A sampling pump, Model CS1 (Casella), with a flow-rate adjustable between 20 and 200 ml min^{-1} was used.

Gas-tight syringes

Syringes of 1 and 20 ml capacity (J. Young, S.G.E.) were used.

Reagents and Standards

Hydrogen sulphide, 99.6%. BDH Chemicals.
Methanethiol, 99.5%. BDH Chemicals.
Ethanethiol, 99.5%. BDH Chemicals.
Dimethyl sulphide, 98%. Aldrich Chemicals.
Dimethyl sulphide, 99%. Aldrich Chemicals.
Calibration gas. 1000 v.p.m. hydrogen sulphide in nitrogen (BOC Special Gases).
Ozone. Model Trailigaz Labo ozone generator.
Ozone measuring tubes. Draeger Safety.

Caution—Hydrogen sulphide and ozone are highly toxic. Thiols and organic sulphides are toxic and flammable. Suitable precautions should be taken at all stages when handling these materials.

Chromatography and Thermal Desorption

The gas chromatograph was set up according to the manufacturer's instructions and the instrument optimised for sulphur analysis. The conventional septum injector was removed and the carrier gas supply taken to the inlet of the thermal desorber. The desorber has two gas outlets that are selected depending on the mode of use; one simply diverts the carrier gas back to the chromatograph where it flows via a switching valve and sample injection valve in series directly to the inlet of the analytical column. In this mode the gas chromatograph is used conventionally and gas samples are introduced by way of the injection valve and a gas-tight syringe.

The other mode diverts the carrier gas through the desorption oven and cold trap and then back to the chromatograph; flow switching is controlled automatically by the desorber, and by the operator. Flows of gas are controlled at all times by metering valves and flow meters.

The gas chromatographic conditions used were as follows: detector temperature, 150 °C; injector temperature, 60 °C; oven temperature, 50 °C; temperature programme, 2 min at 50 °C isothermal, ramp at 15 °C min^{-1} to a final temperature of 110 °C held for 9 min; carrier gas flow-rate, 40 ml min^{-1}; flame gases, hydrogen at 20 ml min^{-1}, air at 30 ml min^{-1}, air purge approximately 100 ml min^{-1}; amplifier attenuation, 32 × 10^2; output to integrator, 1 V f.s.d.; integrator attenuation, × 1024.

The thermal desorber was modified by replacing the stainless-steel cold trap with a trap of the same dimensions made of glass-lined stainless steel, in order to minimise any adsorption of reactive gases. In some experiments, a length of 1.6 mm o.d. PTFE tubing was passed through the trap if a smaller trapping volume was required. In practice either arrangement worked well. No packing was used in the trap as cryogenic re-trapping proved to be quantitative. All transfer pipework in contact with the samples was either 1.6 or 3.2 mm o.d. PTFE tubing and, where it was necessary to use stainless-steel couplings, they were lined with PTFE or silicone-rubber tubing.

An additional ball valve and fine metering valve were fitted in the sample transfer line and a switch was added to override the automatic flow switching sequence of the desorber. The latter allowed the temperature programmed runs to be completed without disturbing the gas flows. Finally, a septum port was added to allow gas injections during optimisation of desorber conditions.

The desorption and re-trapping conditions used for all the sulphur compound studies were as follows: desorption temperature, 110 °C; desorption time, 3 min; cold trap (trapping), −186 °C; cold trap (volatilisation), 110 °C.

A previous study[11] indicated the need for conditioning the chromatographic system during sulphur gas analyses. The whole system was conditioned prior to a set of analyses by injecting 1 ml of 1000 v.p.m. H_2S in nitrogen through the septum port on to the desorber. This conditioned all parts of the system in contact with the desorbed sample, and was necessary only if the system was not used for more than 2–3 h. The column was conditioned immediately before each analysis by injecting 250 μl of 1000 v.p.m. H_2S through the sample injection valve.

Calibration

Dynamic H_2S gas standards

A concentration of 1000 v.p.m. H_2S in nitrogen was serially diluted with air to give standards in the range 0.1–1.0 v.p.m. H_2S. Each mixture was monitored with the hydrogen sulphide monitor to indicate when the mixture was fully equilibrated; with a final flow-rate of 5 l min^{-1} going into the sampling flask, a stable mixture is obtained in 4 min, and where the large chamber was used, at least 20 min were required for equilibration.

Static gas standards

Static gas standards of hydrogen sulphide and the other sulphides and thiols were prepared by injecting suitable volumes of gases and vapours into the large chamber through the septum port.

A knowledge of the saturated vapour pressure of the pure volatile liquids at room temperatures allows semi-quantitative standards to be made up in the range 0.1–0.5 v.p.m. by serially diluting the saturated vapour using gas syringes and septum vials. When injected into the chamber, the various compounds were allowed to mix for several minutes using the built-in fan. After mixing, samples were taken immediately as described in the following sections and, in order to minimise losses due to adsorption, etc., no more than 5 min elapsed between mixing and sampling. After sampling, the chamber was thoroughly flushed with air before preparing new standards. Standards made up in this way were used only to provide a qualitative analysis of compounds other than H_2S.

Calibration procedure

After the preparation of standards by one of the above procedures, samples were taken for gas chromatographic analysis; the sampling technique was identical for either type of standard and, with minor variations, identical with the field sampling method.

A freshly conditioned tube was placed in a polystyrene block. A short length of silicone-rubber tubing, approximately 20 cm, was attached to each end of the absorbent tube, one piece of tubing being used to sample the calibration mixture and the other attached to the inlet port of the sampling pump. Liquid argon was poured into the chamber of the block to completely cover the exposed portion of the adsorbent tube. The tube and block were allowed to chill through for about 1 min, topping up the argon as necessary. Care should be taken in handling the block at this stage, particularly with regard to leaks of argon past the tube. Blocks should be tested before use and any that leak significantly rejected. With careful use, blocks can be used many times before leaks occur.

Tests with a micro-probe thermocouple indicated that a considerable temperature gradient existed both along the adsorbent bed and across it. Temperatures at the centre of the packing dropped to approximately −10 °C, whereas temperatures of −166 °C were recorded at the inner wall of the tube directly in contact with the argon. These temperature gradients were considered beneficial, however, as it was likely that a more even spread of adsorbed and condensed materials occurred, and the tube was less prone to blockages due to icing when ambient levels of water vapour were high.

A sample was collected by switching the pump on for the required length of time. For the purpose of this study, the sampling period was set at 1 min with a previously calibrated flow-rate of 100 ml min^{-1}, which gave adequate sensitivity for 0.1 v.p.m. H_2S. The sensitivity of the method may be changed as required by altering the sampling times or flow-rates within certain limitations. It was found that at least 1000 ml of ambient air of 35% relative humidity could be aspirated through the adsorbent tube before the flow-rate was reduced owing to ice formation, but flow-rates should not exceed 100 ml min^{-1} otherwise trapping will not be quantitative. During aspiration the actual flow-rate was checked by connecting a bubble flow-meter to the outlet of the sampling pump.

When sampling was complete, the pump was switched off, the silicone-rubber tubing removed, argon returned to a Dewar vessel and the tube inserted immediately with forceps into the desorber oven. Approximately 10 s before the tube was inserted into the oven, the automatic cycle of the desorber was started, which allowed the cold trap Dewar to rise into position and thoroughly cool the glass-lined trap. At this stage a ball valve was closed and no flow of carrier gas took place through the adsorbent tube.

When the tube was correctly positioned, the ball valve was opened and a metering valve used to restrict the flow of gas to 4 ml min^{-1} during the desorption and re-trapping. The completeness of re-trapping at this stage was checked by injecting an aliquot of hydrogen sulphide into the system before the cold trap and observing any breakthrough of H_2S on the chromatograph. Under the conditions described, no breakthrough was recorded. The aliquot of hydrogen sulphide used to condition the system was injected through the sample injection valve immediately after placing the adsorbent tube in the oven, and this peak appeared approximately 1 min before the first analytical peak.

At the end of the pre-set desorption period, the cold trap Dewar lowers automatically, and the trap was heated quickly to 110 °C by resistive heating. At this point, the full carrier gas flow was restored by opening the metering valve fully, the integrator and temperature programming being started simultaneously. For hydrogen sulphide and methanethiol, the whole process was completed in less than 7 min, with retention times of 0.6 and 1.5 min, respectively, whereas dimethyl disulphide eluted in approximately 13 min. On the column described, all five sulphur compounds investigated were completely resolved; using dynamic gas standards, a plot of log (peak area) against log (concentration) yields a linear calibration over the range investigated for H_2S.[11] Typical instrument responses for a series of hydrogen sulphide standards are given in Table 1.

Table 1. Hydrogen sulphide dynamic gas standards. Limit of detection based on the total standard deviation at 0.1 v.p.m. = 0.07 v.p.m. H_2S

Nominal hydrogen sulphide concentration, v.p.m.	Mean peak area, counts × 10^6,	Total standard deviation, counts × 10^6
1.00	2.58	0.24 (3)*
0.75	2.22	0.06 (2)
0.50	1.59	0.12 (4)
0.40	1.33	0.08 (2)
0.30	0.94	0.08 (2)
0.20	0.66	0.06 (2)
0.10	0.37	0.04 (6)

* Degrees of freedom in parentheses.

Ozone Removal Investigation

The presence of ozone in some of the field samples was expected to interfere with both sample storage and analysis.

The reaction between ozone and hydrogen sulphide in the absence of water is slow at ambient temperatures, but the technique employed in this investigation included long-term sample storage and thermal desorption, both of which could lead to losses of sulphur compounds through oxidation. It was therefore decided to seek some means of selectively removing ozone prior to trapping the determinands on the Tenax adsorption tube.

The laboratory ozoniser was set up using the ozone production graphs supplied by the manufacturer, and the ozone gas was introduced into the dynamic gas calibration system in such a way that a final mixture containing pure air, hydrogen sulphide and ozone could be manipulated with flow meters to give any combination of gas concentrations in the ranges 0–10 v.p.m. of hydrogen sulphide and 0–50 v.p.m. of ozone. During tests with 10 v.p.m. of ozone and 1 v.p.m. of hydrogen sulphide, it was noted that even with liquid argon cooled adsorption tubes some darkening of the Tenax material took place at the inlet side, which indicated that reaction between ozone and Tenax was taking place, and that the adsorption - desorption characteristics of Tenax so affected would be altered. This was confirmed by placing a Draeger ozone-indicating tube in the sampling line immediately after the Tenax tube and aspirating up to 20 v.p.m. of ozone through the Tenax; under normal sampling conditions, no ozone was detected, it having been adsorbed or reacted with the Tenax. Tests to find an effective and selective ozone adsorbent were conducted by placing the adsorbent under trial in an empty glass adsorbent tube, with glass tubes containing strips of moistened starch - iodide indicator paper before and after the test material, thus giving a semi-quantitative and convenient means of checking the efficiency of ozone removal.

It was observed during tests that where a particular type of silicone-rubber tubing was used to connect the adsorbent and indicating tubes together, ozone appeared to be significantly removed. Further tests showed that the 20 cm length of silicone-rubber tubing used to collect samples, as described under Calibration, was effective in removing ozone up to 10 v.p.m. when used under the sampling conditions of flow-rate, etc. Tests were carried out on a number of other tubing materials of similar dimensions. Synthetic and natural rubber tubing was not effective; nor was poly(vinyl chloride) (Tygon) tubing or certain other types of silicone-rubber such as opaque types. The most effective material that could be easily identified and obtained commercially was a translucent silicone rubber tubing 6 mm o.d., 4 mm i.d., batch number 50318, manufactured by Esco Rubber. The efficiency of the tubing depended on the amount of ozone that it had been exposed to, and its capacity for ozone removal decreased with exposure to ozone, presumably as active adsorption - reaction sites were used up. A fresh length of tubing would reproducibly remove 10 v.p.m. of ozone before the Tenax tube, but attempts to re-activate the tubing by heating or exposure to hydrogen sulphide gas were not successful.

In order to increase the lifetime of the silicone-rubber tubing, tests were carried out with short pre-columns of adsorbants. The columns were made from 80 mm lengths of 6 mm o.d. thin-walled PTFE tubing packed with adsorbent and plugged at each end with glass-wool. Silicone-rubber tubing was dipped into liquid argon and, when brittle, could be filed with a coarse file into a granulated form that was then used to pack the tube; this material proved very effective in removing ozone, and a similar material described as silicone rubber "crumb" was obtained from Esco Rubber. This material was used for all subsequent tests and a fresh PTFE tube containing the crumb was attached to the inlet end of the silicone-rubber sampling tube when sampling for hydrogen sulphide in the presence of ozone. When these disposable tubes were used, it was never necessary to change the silicone-rubber tubing and it was estimated that the capacity of the pre-column was at least 100 ml of sample containing 10 v.p.m. of ozone. The results of a test in which samples of air containing 1 v.p.m. each of hydrogen sulphide and ozone were subjected to immediate analysis and storage for approximately 24 h followed by analysis are given in Table 2.

The results of this test indicated that the finely divided silicone-rubber material was the best adsorbent for ozone and had little effect on the level of hydrogen sulphide after storage. A further check on this material was made by collecting a nominal 1.0 v.p.m. hydrogen sulphide sample on a number of Tenax tubes after passing the sample through a pre-column. Samples were collected both in the absence of ozone and in the presence of 1.0 v.p.m. ozone, and the results of analyses are shown in Table 3.

Comparison of the means by Student's *t*-test indicated no significant difference at the 95% confidence level.

Field Sampling

Field sampling was carried out in a manner identical with that described under Calibration. The only additional equipment required for field sampling was a storage Dewar vessel to transport adsorption tubes from the site to the laboratory, and plastic end-caps to seal the ends of tubes after a sample had been taken. A suitable Dewar vessel should have internal dimensions of approximately 30 cm depth and 1 cm diameter. A container with an internal diameter of 100 mm and a depth of 100 mm was provided. This could float on the surface of the liquid argon and allowed capped tubes to lie horizontally on the bottom of the container, the objective being to maintain the tubes in close contact with the liquid argon. Provided that the argon liquid level was maintained as high as practically possible, the storage temperature of the tubes could be maintained below −150 °C. An efficient Dewar vessel should allow the storage of samples for several days without frequent topping up of argon. During later field sampling, liquid nitrogen (boiling-point −196 °C) was used as the trapping and storage liquid, being approximately one third of the cost of argon, but argon was retained as the cryogenic liquid in the desorber to prevent condensation of the carrier gas.

Table 2. Effect of 1 v.p.m. of ozone on the storage of Tenax adsorbent tubes containing hydrogen sulphide, in the presence of various pre-column materials. Nominal hydrogen sulphide concentration, 1.0 v.p.m. All results quoted are a mean of two or more analyses

Pre-column material	Control (immediate analysis), v.p.m. H_2S	Storage (analysis after 24 h), v.p.m. H_2S
10 cm long PTFE tubing	0.73	0.52
20 cm long silicone tubing	1.02	0.76
Finely divided silicone-rubber pre-column	1.06	1.01
Silicone-rubber crumb pre-column	1.12	0.88

Table 3. Results of the analysis of nominal 1.0 v.p.m. hydrogen sulphide in the presence of 1.0 v.p.m. ozone using a finely divided silicone-rubber crumb pre-column

	No ozone (immediate analysis), v.p.m. H_2S	1.0 v.p.m. ozone (24 h storage), v.p.m. H_2S
Mean H_2S concentration, v.p.m.	1.053	1.027
Number of analyses	6	6
Total standard deviation	0.031	0.075
Recovery, %	—	98

A check on the efficiency of storage was carried out by preparing dynamic gas standards of 0.2 and 1.0 v.p.m. H_2S, analysing one batch of tubes immediately and storing others for periods up to approximately 30 h in the storage Dewar vessel. The results of these tests are given in Tables 4 and 5.

Once a sample had been taken on site, the tube was immediately sealed with standard 6 mm plastic or rubber chromatographic column end-caps that had previously been heated to 100 °C to remove trace amounts of volatile sulphur compounds.

To analyse the capped tubes, once removed from the storage Dewar vessel, a tube was placed in a polystyrene block similar to that used for sampling, with slots cut in the top instead of holes bored through. This allowed a tube to be semi-immersed in liquid argon while the caps were removed. In practice, removal was difficult as the caps shrink tightly on to the glass tube when cooled, and are no longer flexible; however, by rotating the tube carefully with the caps between fingers, it is possible quickly and safely to warm the caps to a point at which they can be removed, whilst keeping the Tenax section cooled. A later refinement was to shorten commercially available caps to half their original length (15 mm), which helped the removal process. As soon as the caps were removed, the tubes were analysed immediately, as described previously.

Results and Discussion

Hydrogen sulphide was the only gas that was analysed using dynamic gas standards; the advantage of dynamic standards is that it can normally be assumed that at the low levels a gaseous component will reach equilibrium with its surroundings, and that losses due to physical processes will be minimised; the disadvantage is that a relatively large supply of gas components is required, preferably in undiluted form. Gas standards can be bought in (although stability would be a problem with reactive gases at low concentrations), or produced by serial dilution of a higher concentration, or by some other means such as a permeation oven. In this investigation, a commercial gas standard of H_2S was used, but further dilutions of between 1000- and 10 000-fold were required to achieve levels between 1.0 and 0.1 v.p.m. H_2S. Errors in serial dilution are cumulative and it was considered that the problem of the production of stable calibration standards was the single most important reason for non-reproducible results and for some of the low recoveries in storage tests. The flow meters and dynamic standards technique used were adequate for the purposes of the application, but it is recommended that for more critical measurements, due regard should be given to the provision of high-quality flow controllers, pressure regulators and control of other factors such as temperature. A permeation oven would overcome some of these difficulties and almost certainly would be essential for the production of dynamic standards of the non-gaseous sulphur compounds.

The results of the storage tests (Tables 4 and 5) show variability in the recoveries obtained at the 1.0 and 0.2 v.p.m. levels, more so at the lower level. It was considered significant for the purposes of the investigation that there was no loss of sample over the storage period, and that once the tubes were installed in the storage Dewar vessel at liquid argon or nitrogen temperatures, it would be unlikely that changes in the levels of adsorbed compounds would take place over storage periods longer than those observed.

As previously noted, the variability observed in this investigation was almost certainly due to the difficulties in producing accurate low levels of the compounds under investigation, and at the 0.2 v.p.m. level, the flow meters used were at the bottom of their usable range. At this level, flows were difficult to reproduce and maintain, and as the calibration standards and storage samples were produced by the same technique, it was concluded that this factor alone would account for variations. It was not possible to investigate this aspect further and the mean recoveries obtained during storage were applied to site samples stored for similar periods.

A second important factor was the manipulation of samples. Calibration adsorption tubes were always placed quickly into the desorption oven, hence minimising losses of determinands, whereas field samples were capped and were subject to varying amounts of manipulation. This was partly confirmed when tubes were sorted without caps at all and gave recoveries as good as tubes that had been capped. The uncapped tubes were removed quickly from the sampling block and placed on the bottom of the storage Dewar vessel inner container, whereas others were placed inside screw-capped test-tubes and immersed directly into liquid argon. It should be noted that liquid argon must not be allowed to enter the sample tubes, as the rapid evaporation of the liquid during manipulation causes severe loss of trapped materials (Table 6). A possible explanation for the good recoveries is that any losses from the tube during storage were balanced by the ease with which tubes could be transferred from the Dewar vessel to the desorption oven without delay.

Storage of tubes, as described under Field Sampling, ensures that the temperature of the adsorbent is kept well below the melting-point of any of the compounds studied, and that losses due to physical processes should be minimal. In practice, it is difficult to maintain a constant low temperature in the tube storage vessel, especially during the removal of tubes for analysis. A possible solution might be to store tubes directly in the liquid argon phase, but it would be necessary to find a means of sealing tubes against the ingress of liquid, and no practical solution to the problem was found that was convenient to use under site conditions.

A brief investigation into the processes involved in the sampling procedure was carried out by using sampling tubes packed only with GC-grade silanised glass-wool, and samples of calibration (H_2S) mixtures collected and analysed by the

Table 4. Mean concentration and recovery of hydrogen sulphide after storage, nominal 1 v.p.m.

Time/h	6	18	24	30
Mean, v.p.m. H_2S	0.83	0.83	0.95	0.99
Total standard deviation	0.06	0.07	0.11	0.05
Number of analyses	4	4	4	4
Recovery, %	83	83	95	99
Mean recovery (all results)	90%			

Table 5. Mean concentration and recovery of hydrogen sulphide after storage, nominal 0.2 v.p.m.

Time/h	6	18	24	30
Mean, v.p.m. H_2S	0.11	0.13	0.14	0.16
Total standard deviation	0.05	0.03	0.06	0.02
Number of analyses	4	4	4	4
Recovery, %	55	65	70	80
Mean recovery (all results)	68%			

Table 6. Comparison of various methods of Tenax tube storage, nominal 0.5 v.p.m. H_2S. Three samples of each tube were analysed

Calibration standard	Mean peaks, counts × 10^6	Total standard deviation	Degrees of freedom	Concentration, v.p.m.
0.5 v.p.m.	1.34	0.16	2	—
Uncapped tubes, 3 h storage	1.64	0.14	2	0.60
Uncapped tubes, total immersion, 3 h storage	1.38	0.23	4	0.51

Table 7. Recovery of hydrogen sulphide gas standards from tubes packed with glass-wool compared with the standard procedure

Standard procedure: H_2S concentration, v.p.m.	Glass-wool procedure		
	Peak area, counts × 10^6	Mean calibrated concentration, v.p.m.	Recovery, %
0.98	3.13	1.10	112
	3.21		
0.79	2.64	0.88	111
	2.65		
0.60	2.06	0.70	117
	2.18		
0.41	1.21	0.40	98
	1.32		
0.21	0.56	0.16	76
	0.56		

Tenax procedure. The results are shown in Table 7 compared with conventional Tenax tube samples. The recoveries and precision are acceptable, although non-linearity is evident. It is clear that the main process involved in sample collection is condensation, assuming that the glass-wool itself has no adsorptive properties and provides only an inert support for the condensed materials. It was considered, however, that the use of Tenax was preferable as this probably combined condensation and adsorption, and no attempt was made to use glass-wool only in any site investigations.

As previously noted in the section dealing with experimental conditions, there is a possibility of extending the sensitivity of the method considerably; there is no evidence that blank or instrument noise levels would be a problem for an increase in sensitivity of several orders of magnitude. The method is potentially useful in the study of other sulphur compounds of environmental interest, such as sulphur dioxide, carbonyl sulphide and carbon disulphide, and by changing the chromatographic or detection system the method offers a convenient means of qualitative or quantitative analysis of non-sulphur compounds.

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Effects of Slow Heating Rates on Products of Polyethylene Pyrolysis

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Polymeric materials, although they are not volatile themselves, may be analysed by gas chromatography (GC) through the use of analytical pyrolysis. Because the fragmentation pattern produced by heating is reproducible, the pyrolysates provide much information about the original macromolecule of which they were a part. Analytical pyrolysis, coupled with GC, mass spectrometry (MS), GC - MS and Fourier transform infrared (FTIR) spectroscopy has provided a wealth of information concerning polymer microstructure, branching, defect structure and degradation mechanisms. Traditionally, the pyrolyses have been carried out at very fast heating rates (for example, heating to 800 °C for 5 s at a rate of 10 000 °C s^{-1}). Recently, however, interest has been shown in how polymers behave when heated to pyrolysis temperatures at slow rates (°C min^{-1}) for long periods of time. Many polymers show considerable shifts in the kinds and relative amounts of pyrolysates produced at these slower rates. The polyolefins are good examples of polymers for which the pyrolysate distribution is sensitive to both the final temperature and the rate of heating. Examples are given indicating these differences for polyethylene in a series of pyrolyses in which the temperature is varied and the heating rate held constant, and for pyrolyses at a variety of heating rates to the same final temperature.

Keywords: *Pyrolysis; gas chromatography; polyethylene; degradation*

Analytical pyrolysis has been widely used in the study of polymers such as the polyolefins. The analysis of polyolefin pyrolysates has done much to elucidate structural anomalies such as branching and defect structures,[1-3] and also to shed light on questions of kinetics and degradation mechanisms.

When the polyolefins are pyrolysed, they fragment to produce volatile hydrocarbons that may be separated and studied using gas chromatography. Polyethylene is essentially a high relative molecular mass wax that degrades thermally to produce smaller hydrocarbon fragments. Most of the volatile compounds produced are unbranched hydrocarbons from the basic polymer chain, but branched compounds are also produced, and are of great importance when studying the degree and type of branching found in the polymer structure.[4]

The unbranched hydrocarbons have double bonds at one or both ends of the molecule. This produces a characteristic pattern consisting of a series of triplet peaks when the pyrolysates are chromatographed. Each triplet is composed of the alkane, alkene and the diene having the same number of carbons. Typically, a pyrogram contains a series of triplets representing hydrocarbons of every chain length to about 30 carbons. Examples of this are shown in Fig. 1, in which the pyrolysates were chromatographed on a 50 m × 0.25 mm i.d. SE-54 fused-silica capillary column. The order of elution here is the alkadiene first, followed by the alkene, with the alkane last. The relative abundance of the alkane and the alkadiene in each triplet is sensitive to both the pyrolysis temperature and the rate of heating used to effect the pyrolysis.

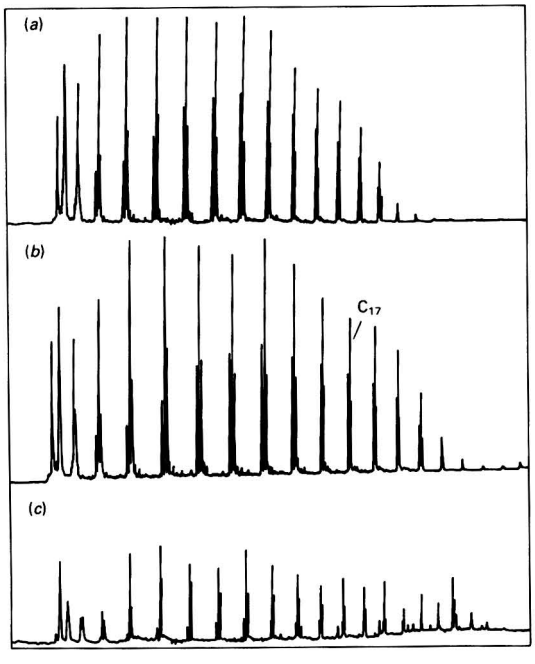

Fig 1. Gas chromatograms showing effect of pyrolysis temperature on polyethylene. Conditions: ramp, 20 °C ms^{-1}. (*a*) Temperature 1000 °C for 20 s, GC programme 50 °C for 2 min, 4 °C min^{-1} to 280 °C. (*b*) 900 °C for 20 s; and (*c*) 700 °C for 20 s

Experimental

All pyrolyses were performed using a filament coil type pyrolyser (Pyroprobe 122, Chemical Data Systems). The polymer samples were held in a quartz tube that was inserted into the platinum coil. Because pyrolyses at slow rates were to be studied, the pyrolyser was interfaced to a sample concentrator (Model 320, Chemical Data Systems), which collected the pyrolysates on a Tenax filled trap. In this way, polymer samples could be processed for minutes or hours at slow heating rates, and the pyrolysates all collected for a single GC analysis. When the pyrolysis was complete, the trap was pulse heated and backflushed with the GC carrier gas. The desorbed pyrolysates were transferred to a gas chromatograph (Model 3700, Varian) equipped with a 50 m × 0.25 mm i.d. SE-54 capillary column (Quadrex). A 60:1 split was established at the injection port of the GC and the column was programmed from 50 to 280 °C at a rate of 4 °C min^{-1}. A flame-ionisation detector was used, and area data were obtained from a recording integrator (3390-A, Hewlett-Packard).

Results and Discussion

The temperature at which a material is pyrolysed has a significant, and often predictable, effect on the nature and amounts of compounds produced. Polyethylene, when pyrolysed at temperatures ranging from 600 to 1000 °C, produces the series of triplets each time [Fig. 1(*a*)–(*c*)] but with some interesting differences. The relative amounts of the saturated and unsaturated products at each carbon number change as the pyrolysis temperature is changed. Specifically, for each triplet, the amount of alkane decreases and the amount of diene increases as the pyrolysis temperature increases. A ratio of the amount of diene to the amount of alkane (*D*/*A*) at specific carbon chain lengths may be calculated, and it is interesting to see how this ratio is affected by the pyrolysis temperature. Fig. 2 shows a graph of the *D*/*A* ratio for compounds of 10–19 carbons plotted against the number of carbons in the chain for pyrolysis temperatures from 600 to 1000 °C. From this graph it can be seen that the diene to alkane ratio increases with temperature, and that the ratio is roughly the same for most diene - alkane pairs having between 13 and 19 carbons. There are some fluctuations, with the *D*/*A* ratio peaking at tetradecane and heptadecane, and dipping slightly at pentadecane and hexadecane, regardless of temperature.

The temperature dependence of this ratio may be seen more easily by selecting a specific number of carbons and comparing the *D*/*A* ratio at this carbon number for various pyrolysis temperatures. For example, the ratio of heptadecadiene to heptadecane could be chosen, and plotted against pyrolysis temperature. Fig. 3 is such a graph, showing that the relationship between the *D*/*A* ratio and pyrolysis temperature is, in fact, linear in the range 600–1000 °C.

The above examples were all pulse pyrolyses, using a rate of 20 °C ms^{-1} to achieve the final temperature. It was of interest to study the converse situation, *i.e.*, pyrolysis to the same final temperature each time, but at different rates of heating. Rates between 5 and 60 °C min^{-1} were chosen, and the pyrolysis products compared with chromatograms of pulse pyrolyses (20 °C ms^{-1}) of the same material.

In order to achieve this, some means of retaining the compounds produced during a slow pyrolysis must be used. For example, if pyrolysis is performed at 5 °C min^{-1}, to a final temperature of 900 °C, the heating process will take 3 h. It was desired to collect all of the volatiles produced during this time period and to inject them on to the GC column for a single chromatogram. For this reason, the CDS Pyroprobe 123 was selected, as it consists of a pyrolyser capable of heating coil or ribbon probes at rates as low as 5 °C min^{-1}, and a sample concentrator that collects the pyrolysis products as they are produced. The unit uses a heated interface (into which the probe is inserted for pyrolysis) that is constantly swept with a stream of helium. This sample stream carries the pyrolysates out of the interface and into a Tenax packed trap, where they are retained while the helium is vented. After the pyrolysis is complete, the trap is backflushed with the GC carrier gas as it is pulse heated to desorb the pyrolysates from the Tenax. The GC carrier flow then takes the pyrolysates through a heated transfer line into the injection port of the gas chromatograph. Instrument parameters are listed in Table 1.

Fig. 4(*a*) shows the material collected from a pyrolysis of polyethylene at 60 °C min^{-1} to 900 °C. Comparing this with Fig. 1(*b*) (pulse pyrolysis at 900 °C for 20 s) shows the significant effect on the *D*/*A* ratio. If the heating rate is decreased to 30 °C min^{-1}, the *D*/*A* ratio is further decreased, and a rate of 5 °C min^{-1} diminishes the *D*/*A* ratio still more, as can be seen in Fig. 4(*b*) and 4(*c*). This decrease in the *D*/*A* ratio as the rate of pyrolysis is decreased (or an increase in the ratio as the rate is increased) mimics what was observed for the *D*/*A* ratio for pulse pyrolyses at various temperatures. In fact, if the rate were not known, or the pyrograms were assumed to be the result of pulse pyrolysis at a very rapid rate, the

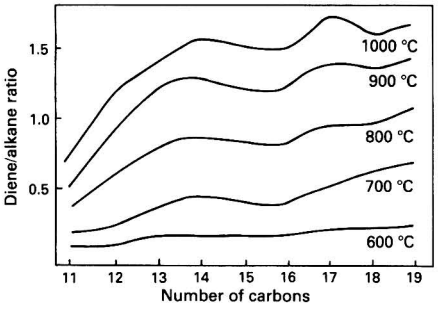

Fig. 2. Pyrolysis of polyethylene at 20 °C ms^{-1}. Diene to alkane ratio *versus* number of carbon atoms

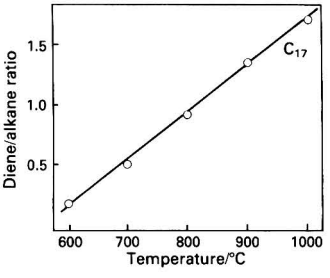

Fig. 3. Pyrolysis of polyethylene at 20 °C ms^{-1}. Diene to alkane ratio *versus* temperature

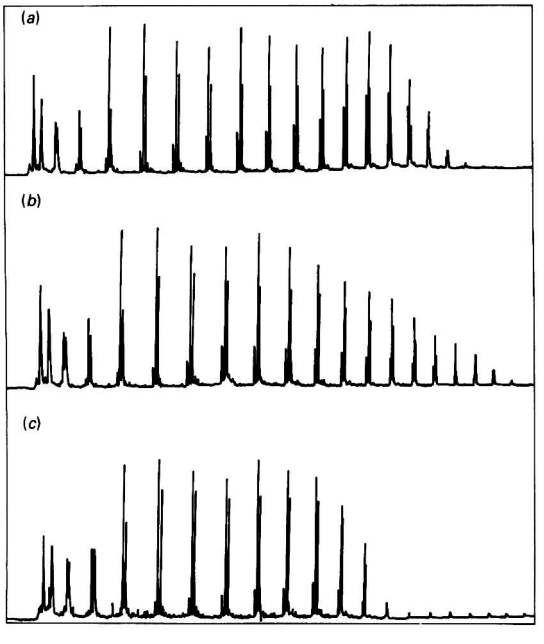

Fig. 4. Gas chromatograms showing effect of pyrolysis rate on polyethylene. (*a*) Ramp 60 °C min^{-1}, temperature 900 °C, GC programme 50 °C for 2 min, 4 °C min^{-1} to 280 °C. (*b*) Ramp 30 °C min^{-1}, temperature 900 °C. (*c*) Ramp 5 °C min^{-1}, temperature 900 °C

Table 1. Operating conditions of the sample concentrator and gas chromatograph

320 sample concentrator—	
Valve oven	275 °C
Transfer line	275 °C
Thermal desorber	250 °C
Trap desorption	275 °C for 2 min
Trap bake	290 °C for 2 min
Sample carrier	30 ml min^{-1}
Trap carrier	30 ml min^{-1}
GC carrier	60 ml min^{-1}
Gas chromatograph—	
Column	50 m × 0.25 mm i.d. SE-54
Initial	50 °C for 2 min
Ramp	4 °C min^{-1}
Final	280 °C
Detector	FID
Carrier	Helium 32 lb in^{-2}

chromatogram would resemble pyrolysis at lower temperatures. A pyrolysis at 5 °C min^{-1} to 900 °C, for example, produces a *D/A* ratio that resembles the products of a pulse pyrolysis at 625 °C. Similarly, 60 °C min^{-1} to 900 °C produces a pattern with a *D/A* ratio that would be expected from a pulse pyrolysis at about 750 °C.

System reproducibility was evaluated through replicate pyrolyses and calculation of the ratios of the areas of peaks for the alkadiene and alkane containing thirteen carbons. At 900 °C this peak area ratio averaged 1.467 ± 0.074, which produces a relative standard deviation of 5.04%.

Conclusions

Both the pyrolysis temperature and the rate at which that temperature is achieved have significant effects on the formation of pyrolysates from a solid polymer. Pyrolyses at very slow rates (°C min^{-1} rather than °C ms^{-1}) produce pyrograms that resemble those of pulse pyrolyses at lower end-point temperatures. It is possible that the slower rates permit one to see more of the primary pyrolysis products, which may be formed at lower temperatures then volatilised and removed from the pyrolysis chamber before secondary pyrolysis reactions ensue. Pyrolyses at elevated temperatures, or at a faster rate to the same final temperature, may enhance secondary pyrolysis reactions. For polyethylene, the general trend is for the production of more terminally unsaturated dialkenes at higher temperatures or faster rates, whereas the corresponding alkane is enhanced at lower temperatures and slower rates.

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Determination in Urine of Diisocyanate-derived Amines from Occupational Exposure by Gas Chromatography - Mass Fragmentography

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Hexane-1,6-diamine was determined in the urine of car painters exposed to paint aerosols based on 1,6-bis(carbonylamino)hexane. The concentration of inhaled functional NCO groups during a 15-min exposure averaged 2.8 ± 0.8 μmol m^{-3} (standard deviation, $n = 5$). The peak diamine concentration in urine of 63 ± 33 nmol (mmol creatinine)$^{-1}$ (standard deviation, $n = 5$) occurred 30 min after the end of exposure. The diamine was extracted from the urine with Sep-Pak silica gel cartridges and determined as the perfluoroamide derivative by capillary gas chromatography - mass fragmentography. The determination of diisocyanate-derived diamines in urine offers a selective and sensitive means of biological monitoring of occupational isocyanate exposure.

Keywords: *1,6-Bis(carbonylamino)hexane; hexane-1,6-diamine; isocyanates; urinalysis; occupational exposure*

Organic diisocyanates are used in, *e.g.*, foams, synthetic rubber, adhesives and paints. Articles made of polymers of diisocyanates (polyurethanes) combine outstanding material properties with economic processing costs for various applications. The main isocyanates in industrial use are the aromatic diisocyanates, bis(carbonylamino)toluene (a 2,4 - 2,6-isomeric mixture) and 4,4′-bis(carbonylamino)diphenylmethane. In addition to the monomeric isocyanates, various isocyanate-terminated pre-polymers derived from monomeric sub-units have been introduced.[1] These have higher relative molecular masses and, consequently, lower vapour pressures. One such polyisocyanate is the biuret structure of 1,6-bis(carbonylamino)hexane. This oligomer is the major building block in two-component polyurethane spray paints used in car paints and many other coatings. The isocyanate component usually contains less than 1% of the volatile monomer.[2]

Conjugated diamines from the percutaneously absorbed monomeric aromatic isocyanate were excreted in the urine of rats.[3] Likewise, the inhalation of vapour of monomeric 4,4′-bis(carbonylamino)diphenylmethane caused dose-related 4,4′-bis(amino)diphenylmethane excretion in urine.[4]

In this paper we describe a novel gas chromatographic - mass fragmentographic method for the determination of exogenous aliphatic diamines in urine. Extracts of acid hydrolysed samples were purified on Sep-Pak silica gel cartridges. The isolated diamine was converted into its perfluoro-substituted amide and determined by capillary gas chromatography - mass fragmentography. The peak hexane-1,6-diamine concentration in urine occurred 30 min after the end of the exposure.

Experimental

Analysis of Isocyanates

Isocyanates were sampled from air on reagent impregnated glass-fibre filters[2,5] for 2–15 min at a flow-rate of 1 l min^{-1}. A 300-mg amount of *N*-[(4-nitrophenyl)methyl]propanamine hydrochloride (Regis Chemicals, Morton Grove, IL, USA) was dissolved in 25 ml of distilled water. Sodium hydroxide solution (15 ml, 1 M) was added, and the mixture was extracted twice with 25 ml of hexane (HPLC grade). A 10-fold dilution of the combined extracts with hexane dried over sodium sulphate gave a 2.6×10^{-3} M impregnation solution. A 20-ml volume of this solution was transferred into a beaker with 20 glass-fibre filters. The filters, without binders and 13 mm in diameter, were obtained from Millipore (Bedford, MA, USA). The beaker was protected against UV light and the solvent was evaporated with a gentle stream of nitrogen. Evaporation was continued until the filters no longer clung to the beaker. The dry filters were placed in 13 mm filter holders (Swinnex, Catalogue No. SX0001300, Millipore). The filters were stored in the dark at −20 °C and were stable for at least 4 weeks. Reference standards were prepared by adding known amounts of the respective isocyanate dissolved in dichloromethane on to the filters. 1,6-Bis(carbonylamino)hexane was purchased from Fluka (Buchs, Switzerland) and the biuret oligomer from Bayer (Leverkusen, FRG). The standard and sample filters were placed in tubes containing 1 ml of the chromatographic solution, shaken for 15 s and then centrifuged.

A 100 μl volume of this solution was injected into a Kontron Model 6000 liquid chromatograph operated with a Uvicon 720 LC variable-wavelength detector at 275 nm. A Hypersil ODS 5 μm, 125 × 4.6 mm i.d. column was used for reversed-phase chromatography. The urea derivatives of the isocyanates were eluted isocratically at 1.5 ml min^{-1} with acetonitrile - water - triethylamine (64 + 35 + 1) at pH 3.

Determination of Hexane-1,6-diamine in Urine

Urine aliquots (5 ml) were heated with 200 μl of concentrated sulphuric acid at 100 °C for 1 h to hydrolyse the acetylated diamine conjugates.[3] The pH of the cooled hydrolysate was adjusted to 9.0–9.3 with saturated sodium hydroxide solution. The sample was loaded on to the Sep-Pak silica disposable column (Waters Associates, Milford, MA, USA). The cartridge was rinsed with 5 ml of water and the diamine was eluted with 5 ml of 0.05 M sulphuric acid. The first 2 ml of the eluate were discarded. The diamine was recovered from the last 3 ml by extraction with 1 ml of toluene after the addition of 1.5 g of sodium chloride and 6 ml of saturated sodium hydroxide solution. The organic phase was removed and 20 μl of heptafluorobutyric anhydride (HFBA) (Pierce Chemicals, Rockford, IL, USA) were added. The mixture was shaken for 30 s, and after 20 min the excess of derivatisation reagent was removed by extraction with 1 ml of 1 M dihydrogen phosphate

buffer (pH 7). The toluene layer was removed and dried over sodium sulphate.

The diamine was determined by capillary chromatography - mass fragmentography. A Hewlett-Packard 5890A capillary gas chromatograph was linked to a Hewlett-Packard 5970A quadrupole mass selective detector. Ionisation was achieved by electron impact (70 eV) and mass spectra were recorded using full (*m/z* 15–550) or multiple ion monitoring (*m/z* 226 + 339). A fused-silica column, 25 m × 0.2 mm i.d., coated with 5% phenylmethylsilicone (cross-linked, Hewlett-Packard) was coupled directly to the ion source. A linear temperature gradient from 115 to 210 °C at 10 °C min^{-1} was used. The carrier gas was helium with a linear gas velocity of 27 cm min^{-1}. The injector was operated in the splitless mode with an inlet temperature of 220 °C and splitless time of 1.5 min. The heptafluoroacyl derivative of hexane-1,6-diamine eluted at 169–170 °C.

Aliquots of a standard solution of hexane-1,6-diamine in 0.05 M sulphuric acid were added to control urine samples and analysed together with the actual specimens. These standards were used for quantitation. The hexane-1,6-diamine results were corrected for the excretion of creatinine determined by the alkaline picric acid method.

Exposure Conditions and Sampling

The 1,6-bis(carbonylamino)hexane-based paint was nebulised by means of compressed air in an exposure chamber (9.7 m^3) for approximately 30 s. The average concentration of the oligomer was 34% and that of the monomer 0.24% in the hardener.[2] After nebulisation, air samples in the closed chamber were collected on reagent-impregnated glass-fibre filters in the breathing zone of five car painters exposed for 15 min.[2] Urine voided 30 min to 8 h after the exposure was sampled and immediately deep-frozen and stored at −25 °C until analysis.

The average isocyanate exposure during a work shift in the paint shop was determined for one car painter. The painter used a respirator with a compressed air supply. Air samples were collected both outside and inside the respirator. Urine voided after the work shift was analysed.

Results and Discussion

Sep-Pak cartridges of silica gel and C_{18} have been used previously for sample clean-up of biological fluids for quantitative analysis by gas or liquid chromatography.[6,7] The aliphatic diamine was extracted from urine using a silica gel cartridge in this study. The procedure is a modification of that described by Brossat *et al.*[6] The clean-up of urine samples was much more efficient with the silica gel cartridge than the acid back-extraction procedure used in our previous studies.[3] The more effective purification of the sample is needed for the gas chromatography with the electron-capture detection. The silica gel step did not affect the over-all recovery, which was 82 ± 7% (standard deviation, $n = 9$). The recovery of hexane-1,6-diamine depended strongly on the alkalinity of the extraction mixture, *i.e.*, 2 ml of saturated sodium hydroxide solution per 1 ml of 0.05 M sulphuric acid were needed to obtain an acceptable recovery. The same effect was shown in an earlier study for the aromatic toluenediamine.[8]

The total ion chromatogram of the heptafluoroamide of hexane-1,6-diamine is shown in Fig. 1(*b*). The acylated derivative was synthesised using a standard procedure for symmetric diamides.[9,10] The molecular ion of *m/z* 508 appeared with a weak relative abundance, 2.8%, in the electron-impact spectra [Fig. 1(*a*)]. The base peak of *m/z* 226 is formed when the carbon chain is split leaving the fragment $C_3F_7CONHCH_2$. The ions of *m/z* 69 and 169, corresponding to CF_3 and C_3F_7, were obtained with high relative abundances (75 and 46%), but as they are not characteristic of the parent

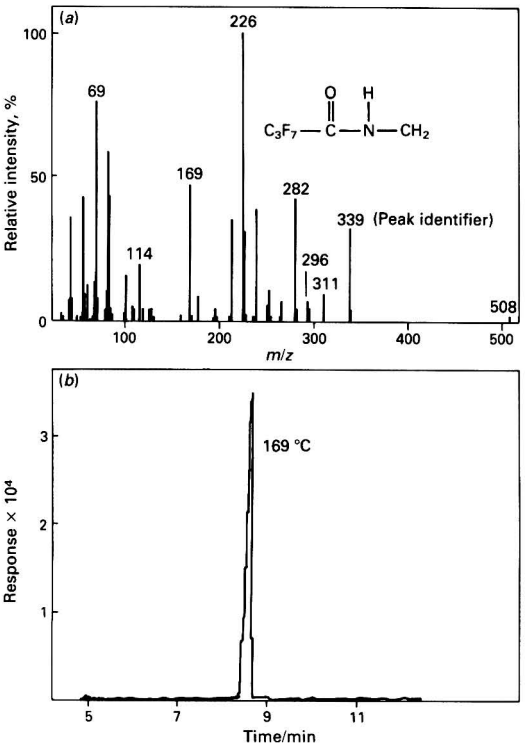

Fig. 1. (*a*) Mass spectra and (*b*) total ion chromatogram of HFBA derivative of hexane-1,6-diamine (115 ng μl^{-1}). The ions correlate to the following presumed fragmentation: *m/z* 508 ($M^{+\cdot}$); 339 (M − C_3F_7); 311 (M − C_3F_7CO); 296 (M − C_3F_7CONH); 282 (M − $C_3F_7CONHCH_2$); 226 (M − $C_3F_7CONH(CH_2)_5$; 169 (C_3F_7); 114 (M − $2C_2F_7CO$); 69 (CF_3). Conditions: column, fused silica, 25 m × 0.2 mm i.d., coated with 5% phenylmethylsilicone; carrier gas, helium, 27 cm min^{-1}; injector, splitless, 1.5 min at 225 °C; and column linear temperature gradient, from 115 to 210 °C at 10 °C min^{-1}

molecule, these fragments cannot be used for the peak identification. The residual ion of *m/z* 282, with a relative abundance of 43%, formed when *m/z* 226 is split off, could be used to obtain structural information. However, it is close to *m/z* 281, the impurity peak of septum or silicone methyl coatings, and is therefore not recommended for the identification. Hence, the ion of *m/z* 339, produced when C_3F_7 is split off, was chosen as the peak identifier. It appeared with a relative abundance of 32%.

The quantification was effected by mass fragmentography of the base peak of *m/z* 226. Peak areas were integrated and the limit of detection, defined as the minimum amount of diamine injected with more than 40 integrator counts, was 0.2 pmol. A 5-fold concentration of the urine sample was achieved by the purification on the silica gel cartridge resulting in a detection limit of 2 nmol (mmol creatinine)$^{-1}$. The base line for the control showed only noise and the integration over the region spanned by the amine peak gave no more than 10 integrator counts. The calibration graph for the perfluoroacyl derivative was linear ($y = 2.65x - 26.82$, where y is area counts and x the amine concentration in ng ml^{-1}) over the range 20–900 pg with a 1-μl sample injected ($r = 0.997$).

The urea derivative for isocyanate determinations is well established and has been tested for several diisocyanates, including the aliphatic 1,6-bis(carbonylamino)hexane and its oligomer with the biuret structure.[2,5]

Hexane-1,6-diamine derived from the parent isocyanate was demonstrated in workers after aerosol exposure for

Table 1. Concentration in urine of amine derived from diisocyanate after aerosol exposure for 15 min

Subject	Exposure level/ μmol NCO m^{-3}*	Concentration of hexane-1,6-diamine/ nmol (mmol creatinine)$^{-1}$ after exposure for: 30 min	4 h	6 h	8 h
1	2.8	16	13	< 2	<2
2	4.0	110	37	9	<2
3	2.2	49	56	< 2	<2
4	3.4	48	25	< 2	<2
5	1.8	90	48	< 2	<2

* Derived from 1,6-bis(carbonylamino)hexane.

15 min (Table 1). The peak concentration of the amine occurred 30 min after the end of the exposure. The concentration of the inhaled functional NCO groups in the breathing zone, originating from 1,6-bis(carbonylamino)hexane, varied from 1.8 to 4.0 μmol m^{-3}.

The spray chamber in the car paint shop was approximately 85 m^3 in size and had roof to floor exhaust ventilation with a nominal air exchange rate of 11.5 m^3 min^{-1}. The average isocyanate monomer concentration in the work-room air was 0.2 μmol m^{-3}. No monomer was detected inside the air hood of the painter, and no hexane-1,6-diamine was observed in his urine sample.

Conclusions

Amine metabolites have been identified in the urine of painters exposed to 1,6-bis(carbonylamino)hexane. The gas chromatographic technique coupled with mass fragmentography allowed the detection of the isocyanate-derived amine in the 0.2 pmol μl^{-1} range.

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Determination of Organic Sulphides by Enthalpimetry Using Chromyl Chloride

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Thermometric titration with chromyl chloride and direct injection enthalpimetry with the same reagent have been used for the determination of sulphides in hydrocarbon solvents; the second procedure was found to be superior. Chromyl chloride and sulphides react in a 1 : 1 ratio, producing a brown precipitate, the heat of reaction being 250 kJ mol^{-1}. The limit of determination is 3 p.p.m. and the relative standard deviation 3.3% at 60 p.p.m. of sulphide-sulphur. The method can be applied in the presence of unsaturated compounds and polycyclic aromatic hydrocarbons. Polycyclic aromatic hydrocarbons also react with chromyl chloride, but the reaction with sulphides is dominant. The method has been suggested for the determination of sulphide-sulphur in petroleum products.

Keywords: *Chromyl chloride; enthalpimetry; sulphide-sulphur; petroleum products; thermometric titration*

In spite of a variety of methods for the determination of sulphides in petroleum products[1–3] the analysis of real samples from petroleum refineries can present great difficulties. Recommended methods based on the oxidation of sulphides to sulphoxides give high results. Ultraviolet spectrophotometry of complexes with iodine is not particularly useful for the direct measurement of sulphides in petroleum samples.

In our efforts to determine sulphides using an enthalpimetric approach, we have examined several chemicals that react easily with sulphides, *e.g.*, chlorine, nitrogen trichloride, chloramine T and chromyl chloride, and we have found chromyl chloride to be the most satisfactory.

Chromyl chloride[4] is readily formed by the reaction of hydrogen chloride with chromium(VI) oxide:

$$CrO_3 + 2HCl = CrO_2Cl_2 + H_2O$$

In the presence of aliphatic hydrocarbons, a red solution is formed. It has been found that in the presence of sulphides, chromyl chloride gives slightly soluble complexes and the heat evolved can be evaluated by enthalpimetry.

Experimental

Apparatus

The apparatus used for the enthalpimetric analysis was as described in the literature.[5,6] The 60-ml reaction cell is placed in a 250-ml Dewar flask. In the lid of the reaction cell are fixed two thermistors, a stirrer and a PTFE 2-mm diameter tube to act as an inlet for the reagent and calibration heater. The enthalpimetric apparatus also includes a regulated, variable, low-voltage power supply, a digital voltmeter, a timer for the calibration heater, a temperature measuring bridge and recorder. The reagent stream is produced by means of a 338 B variable-speed dispenser, made in Poland.

Chemicals and Reagents

The samples of sulphide were purchased from Aldrich Chemie, Steinheim, FRG. Hexane and octane were of analytical-reagent grade.

The solution of chromyl chloride in octane is prepared by shaking 5 g of chromium(VI) oxide, 10 ml of concentrated hydrochloric acid, 3 ml of concentrated sulphuric acid and 250 ml of octane. The clear red organic layer is separated and stored at 0 °C in a brown flask for not longer than 3 d. As the solution is decomposed by water to form chromic acid, its concentration (*ca.* 0.1 M) can be determined by titration with sodium thiosulphate in the presence of potassium iodide and sulphuric acid. The solution is then diluted with octane as required.

Qualitative Analysis

The solution of chromyl chloride in octane can be used for the detection of sulphides and other compounds by adding 1 ml of the reagent to 5 ml of sample and noting the change in colour. Sulphides and disulphides change the colour to brown and in more concentrated solution a brown precipitate is formed. The limit of detection is 5 p.p.m. for sulphide-sulphur and 50 p.p.m. for disulphide-sulphur. The monocyclic aromatic compounds change the colour to brown but no precipitate is formed. The limit of detection in this instance is only 2%. Polycyclic aromatic homo- and heterocyclic compounds such as anthracene, phenanthrene, pyrene and thianthrene change the colour to black and form black precipitates. The sample remains clear but the colour changes to yellow if compounds with active hydrogen are present.

Quantitative Analysis

Two procedures for the determination of sulphides have been examined, one based on a thermometric titration with chromyl chloride solution and the other on direct injection enthalpimetry of sulphides into an excess of chromyl chloride solution.

Procedure 1. *Thermometric titration*

A 40-ml hydrocarbon sample containing 20–150 μmol of sulphide and not more than 25% of monocyclic aromatic hydrocarbons is placed in the reaction cell and the lid is fixed. After stirring for a few minutes at constant temperature, the 0.6–0.8 M solution of chromyl chloride in octane is added with a constant flow-rate of 1–2 ml min^{-1} until the end-point can be determined on the graph indicated by the recorder.

Procedure 2. *Direct injection enthalpimetry*

A 0.02 M solution of chromyl chloride in octane is placed in the reaction cell and the lid fixed. After stirring for a few minutes at constant temperature the 0.5–2-ml samples are injected into the cell, followed by washing with 0.5 ml of hexane. Another 1 ml of hexane is added in order to determine the heat of dilution, which is subtracted from the result for the total volume of the sample.

Results and Discussion

Typical thermometric titration curves for sulphides are shown in Fig. 1. All curves have a sharp beginning and end-point and are similar for different sulphides. In the blank sample the addition of chromyl chloride solution results in a heat evolution of *ca.* 26 kJ per mole of CrO_2Cl_2, which can be assumed to be the heat of dilution. When the reaction with the sulphides is complete, the excess of chromyl chloride evolves the heat of dilution, but the value is not constant and decreases with increasing amounts of sulphides. The results for the determination of the heats of reactions and molar ratios found for different sulphides are summarised in Table 1. From an analytical point of view, it is an advantage that the molar heats of reaction are essentially, within the limits of error, very similar and can be taken as 250 kJ mol^{-1}. Dithiane behaves as a simple sulphide and the second sulphur atom is not indicated.

The next point of analytical importance is that the molar ratio of chromyl chloride consumed to sulphide may be assumed to be unity. The consumption of chromyl chloride calculated from the time of reaction, flow-rate and concentration of reagent is not as useful and sensitive as the heat of reaction, but it may be involved in the analysis of complex mixtures.

The mean relative standard deviation for the thermometric titration of sulphides at 60 p.p.m. of sulphide-sulphur can be accepted as 3.3%, compared with 6% calculated for consumption.

The problem of selectivity is discussed on the basis of results shown in Fig. 2. Consider line A. In spite of a 140-fold excess of hexene with respect to diethyl sulphide, the end-point is still determinable and the result is in agreement with the expected value. The more active cyclohexene is tolerable only at excesses of less than 14-fold. Line C shows the titration of anthracene. It resembles the titration curves of sulphide. However, on the addition of 56 μmol of diethyl sulphide, a distinct end-point corresponding to the added sulphide can be observed. The result this time is too high and a correction should be introduced. The lines E and F demonstrate the titration of phenanthrene and phenanthrene with added sulphide. No shift of the end-point can be observed. Consequently, when dealing with an unknown sample, it may be recommended that the enthalpimetric titration is repeated with some sulphide added.

Table 2 gives the heats of reaction determined by the injection of sulphides into the solution of chromyl chloride given in Procedure 2. The heat of reaction is independent of the type of sulphide and is equal to 300 kJ mol^{-1}, with the exception of diphenyl sulphide. The increase in the heat of reaction from 250 to 300 kJ mol^{-1} may be explained by assuming that in the presence of a great excess of chromyl chloride solution, complexes of the composition $R_2S(CrO_2Cl_2)_2$ are formed.

Disulphides react with chromyl chloride, but the direct titration (Procedure 1) does not give a sharp end-point. The heat of reaction can be conveniently determined using Procedure 2. Table 3 and Fig. 3 demonstrate the combined

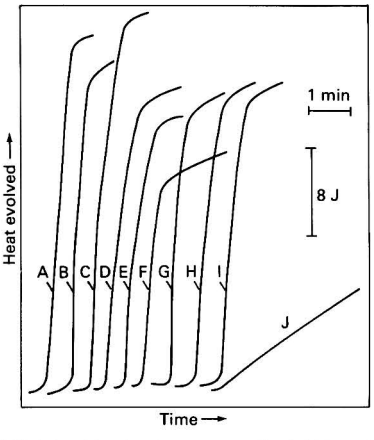

Fig. 1. Enthalpimetric titration curves of sulphides with 0.08 M chromyl chloride in hexane. Flow-rates: A, B, F, G, H and I, 2 ml min^{-1}; C, D, E and J, 1.5 ml min^{-1}. A, Dimethyl 135; B, diethyl 111; C, dibutyl 137; D, di-*sec*-butyl 110; E, di-*tert*-butyl 99; F, didodecyl 80; G, diallyl 101; H, dibenzyl 112; I, 1,3-dithiane 106 μmol; J, blank

Table 1. Molar heats of reaction and mole ratio as determined by the thermometric titration of sulphides with chromyl chloride solution, ± standard deviation (n = 5)

Sulphide	Heat of reaction/ kJ mol^{-1}	Molar ratio, CrO_2Cl_2/R_2S
Dimethyl	243 ± 8	0.97 ± 0.07
Diethyl	266 ± 8	1.02 ± 0.05
Di-n-butyl	254 ± 8	0.99 ± 0.06
Di-*sec*-butyl	251 ± 10	1.05 ± 0.08
Di-*tert*-butyl	248 ± 11	1.05 ± 0.05
Di-n-dodecyl	240 ± 8	0.99 ± 0.07
Diallyl	258 ± 9	1.01 ± 0.05
Dibenzyl	247 ± 8	1.02 ± 0.07
1,3-Dithiane	254 ± 9	1.04 ± 0.08

Table 2. Molar heats of reaction determined by direct injection enthalpimetry of sulphide into an excess of chromyl chloride solution, ± standard deviation (n = 5)

Sulphide	Amount taken/ μmol	Heat of reaction/ kJ mol^{-1}
Diethyl	22	298 ± 10
Di-n-butyl	12	312 ± 17
Di-n-butyl	24	302 ± 11
Di-n-dodecyl	24	298 ± 9
Diphenyl	120	118 ± 8
1,3-Dithiane	28	301 ± 12
Di-n-butyl disulphide	25	407 ± 19

Table 3. Combined analysis of petroleum products

Sample	Sp. gr.	Heat evolved with chromyl chloride/J ml^{-1}: Titration (Procedure 1)	Heat evolved with chromyl chloride/J ml^{-1}: Excess (Procedure 2)	Sulphur content, % *m/m*: Total*	Sulphur content, % *m/m*: KBr - $KBrO_3$ titration	Sulphur content, % *m/m*: Chromyl chloride: Titration (Procedure 1)	Sulphur content, % *m/m*: Chromyl chloride: Excess (Procedure 2)
Kerosene I	0.774	0.54	32.8	0.006	0.32	0.009	0.45
Kerosene II	0.692	2.85	3.70	0.055	0.055	0.055	0.057
Kerosene III	0.783	9.75	22.6	0.15	0.29	0.14	0.31
Diesel fuel	0.843	20.3	200	0.35	0.90	0.31	2.52
Crude oil	0.853	115	242	1.86	—	1.73	3.03

* Determined by combustion.

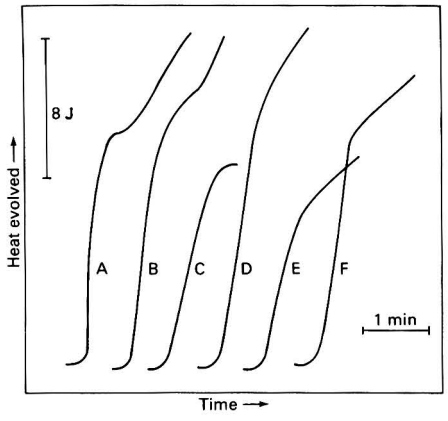

Fig. 2. Enthalpimetric titration curves of sulphides with chromyl chloride in the presence of interfering substances. A, 56 μmol of diethyl sulphide plus 7850 μmol of hexene; B, 56 μmol of diethyl sulphide plus 800 μmol of cyclohexene; C, 22 μmol of anthracene; D, 56 μmol of diethyl sulphide plus 22 μmol of anthracene; E, 22 μmol of phenanthrene; and F, 56 μmol of diethyl sulphide plus 22 μmol of phenanthrene

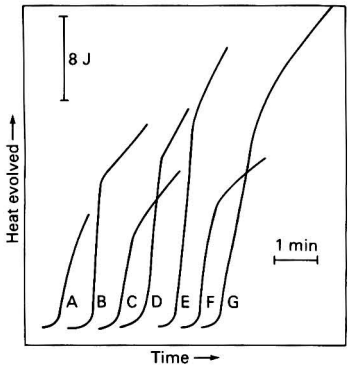

Fig. 3. Enthalpimetric titration curves of petroleum products with chromyl chloride. Total volume 40 ml diluted with hexane. A, 5 ml of kerosene I; B, 5 ml of kerosene II; C, 1 ml of kerosene III; D, 2 ml of kerosene III; E, 1 ml of diesel fuel; F, 0.1 ml of crude oil; and G, 0.2 ml of crude oil

results of the analysis of some petroleum products, including diesel fuel and crude oil. The samples of known sulphur content determined previously by combustion were titrated with a bromide - bromate solution as described for determination of sulphides,[1] and were analysed using Procedures 1 and 2. The sulphur content was calculated from the equations

$$S_1 = \frac{3.2\,Q_1}{250\,d}$$

$$S_2 = \frac{3.2\,Q_2}{300\,d}$$

where Q_1 and Q_2 are the heats of reaction determined by Procedures 1 and 2 and d is the density.

The sulphur content determined by thermometric titration contributes 88–100% of the total sulphur. The difference can be assumed to be due to thiophene and elemental sulphur, which do not react with chromyl chloride. The consumption of bromine gives results that are evidently too high, with one exception. There must be other substances present that react with bromine, apart from sulphides. The highest sulphur content is found by using Procedure 2, indicating the presence of substances that react with chromyl chloride but not with bromine. The difference can be assumed to be due to polycyclic compounds. Consequently, it may be expected that the combined analysis of petroleum will provide some new important information.

It may be added that chromyl chloride has been used for the separation of sulphur compounds from hydrocarbons followed by chromatographic examination.[7]

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Description of Air Pollution by Means of Pattern Recognition Part 2*

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Based on meteorological observations and hourly measurements of chemical constituents at various locations in the city of Schiedam in the Rhine-mouth area near Rotterdam, The Netherlands, a learning machine has been constructed for the description and prediction of complaint situations for polluted atmospheres. It is demonstrated that a seven-parameter model may classify about 80% of the complaint and between 50 and 60% of the non-complaint situations correctly. Sometimes a prediction of a complaint situation up to 6 h in the future is possible, but different meteorological conditions may hamper the predictive ability of the learning machine.

The conditional probability describing the burden on the population in the area runs parallel with the reaction of the human nervous system to an exposure of increasingly concentrated noxious smelling air in test panel experiments. It is shown that the seriousness of the burden influences the total number of complaints filed within a certain period of time.

Keywords: *Pattern recognition; environmental control; Bayes classification; air pollution*

In a previous paper[1] a method was developed to describe an air pollution problem by means of pattern recognition. This was shown to yield only moderate success when the weather conditions were stable; unstable weather conditions gave no success at all. Using this method, a learning machine was constructed based on the stability of the weather, SO_2 concentrations measured at three different locations in the area under investigation and concentrations of hydrocarbons (saturated and olefinic) and ozone. This learning machine was based on 116 hourly reports between 10.00 and 18.00 h in a period from April to June 1979. In this period, no missing features were encountered, the weather was stable and about equal numbers of complaint hours and non-complaint hours were registered. The measurements were made in the Rhine-mouth area near Rotterdam, The Netherlands (Fig. 1).

In this learning machine a sharp separation between the two categories of patterns was seen. However, owing to the severe limitations mentioned, this learning machine could not be used as a predictor of burden on the inhabitants of the area.

In order to improve the predictions, a search was made for better descriptors, yielding the following criteria: a complaint hour was denoted as valid after consideration of the previous 2 h. When in one of these two hours a complaint was reported, the complaint hour itself was included in the training set. In order to combine this category with an about equally populated category of non-complaint hours, for each complaint hour a non-complaint hour was selected 24 h before or after the complaint. According to this procedure daily or seasonal effects were eliminated. Apart from this, another restriction was posed on the non-complaint hour: there should not be any complaint in the period between 5 h before and 5 h after the selected non-complaint hour. The training set was tested by hours taken from measurements in 1981 and 1982.

It was learned from experience that sometimes a complaint was communciated 1–2 h after the burden was noticed. The concentrations of chemical compounds in air exhibit autocorrelation functions with characteristic decay times between 2 and 4 h. Because of these two observations, it was felt that not only were the actual concentrations measured at a given instance of importance, but also the feature values in the immediate past (some hours before).

Another consideration that was taken into account was the logarithmic response of the human nervous system to stimuli from the outside, which means that an increase in burden caused by noxious smells should be rated on a logarithmic rather than on a linear scale.

These considerations dictated the feature transformations as listed below (Practical Considerations). Thus a learning machine was constructed that could serve as a classifier with more success than that published earlier.[1]

Practical Considerations

According to the criteria mentioned in the Introduction, for the year 1982 a total number of 499 complaint hours and 8261 non-complaint hours was found (see Appendix for statistical information). From this set a training set was selected consisting of 35 non-complaint patterns and 38 complaint patterns.

The features describing the learning machine are SO_2 concentrations measured at four different locations, wind direction and the time dependence of two SO_2 concentrations. The SO_2 concentrations were transformed according to

$$(SO_2)_{new} = \ln[SO_2(x) + SO_2(x - 1)]$$

where x represents the hour of observation. The resulting values were autoscaled. The direction of the wind was rated 1 when coming from the industrial area (between south and west) and 0 otherwise. This rating was applied for the hour of observation and the previous 4 h, which gives a rating from 0 up to and including 5. The evolution in time of SO_2 concentrations at a particular location is given by

$$dSO_2/dt = \ln\{[SO_2(x) + SO_2(x - 1)]/[SO_2(x - 2) + SO_2(x - 3)]\} \quad (1)$$

These feature transformations were found empirically, but showed the statistically relevant advantage of a better skewness and kurtosis in comparison with the non-transformed features. Thus a better correspondence with a normal distribution function is found. It was also noticed that the weight of the features for the class separation increased (Table 1, variance weights).

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Results and Discussion

The pre-processed features, logarithmised (except the wind direction) and autoscaled, were weighted (Table 1) and combined according to the Karhunen - Loève transformation. A set of orthonormal eigenvectors was obtained, from which the two with the highest eigenvalues were selected (Table 2). These two eigenvectors, in which the SO_2 concentrations at various locations contribute highly, span a plane in which about 72% of the information is accounted for.

On this plane the pattern space is projected, including the patterns of the test set (Fig. 2). It is seen that an area with only complaint hours (at the left) is separated from an area with only non-complaint hours by an area where both categories are found. This is an indication of the probability distribution that describes the situation.

In such a situation, the non-linear classification according to the nearest neighbour method may give an indication of the quality of the learning machine. The results for such a classification for five nearest neighbours are given in Table 3. Table 3 is based on a Euclidian distance matrix in the seven-dimensional feature space spanned by the features listed in Table 2. It is seen that the non-complaint hours (77% correctly classified) are less predictable than the complaint hours (87% correctly classified). These classifications alter only a few per cent. when less nearest neighbours are consulted. The results of a test set, obtained in the same manner, are listed in Table 4. The classification is only to a minor extent less satisfactory than that of the learning machine itself.

In order to show the ability of this learning machine for the prediction of complaint situations hidden in the near future, a test set of 25 successive hours (May 17th 1982, from 01.00 to 24.00 h) is projected on the application of the same mathematical process as used for the learning machine (Fig. 3). Here it is demonstrated that at the beginning of the observation period, the hours are located in the non-complaint area. It is shown by the drawn lines that gradually the successive hours move towards the region where complaint hours should be expected and indeed two complaint hours are

Table 1. Comparison of variance weights for the two category separation of direct and of logarithmised features

	Variance weights†	
Feature (x_j)*	x	Lnx
$dSO_2(10)/dt$	—	1.39
$dSO_2(11)/dt$	—	1.39
$SO_2(10)$	1.77	2.34
$SO_2(11)$	1.77	2.25
$SO_2(12)$	1.30	1.68
$SO_2(13)$	1.36	1.67

* In the notation $SO_2(y)$, y denotes the sampling location (see geographical map).

† Calculated for the two-category situation 1 and 2 according to

$$W_{j,1,2} = \left\{ \frac{\tilde{x}_{j,1}^2}{N_1} + \frac{\tilde{x}_{j,2}^2}{N_2} - 2\,\frac{\tilde{x}_{j,1}\tilde{x}_{j,2}}{N_1 N_2} \right\} / (\sigma_{j,1}^2 + \sigma_{j,2}^2)$$

where $\tilde{x}_{j,i}^2$ denotes the expectation value for the square of feature j in category i, N_i the population of category i, $\tilde{x}_{j,i}$ the expectation value of the feature j in category i and $\sigma_{j,i}^2$ its variance.

Table 2. Karhunen-Loève transformation of logarithmised features

	Eigenvector, %	
Feature	First	Second
Sulphur dioxide (10)	34	0
Sulphur dioxide (11)	26	32
Sulphur dioxide (12)	11	21
Sulphur dioxide (13)	10	36
Direction of wind	2	4
$dSO_2(10)/dt$	11	4
$dSO_2(11)/dt$	6	3
Eigenvalue (= information)	58.3% + 13.4% = 71.7%	

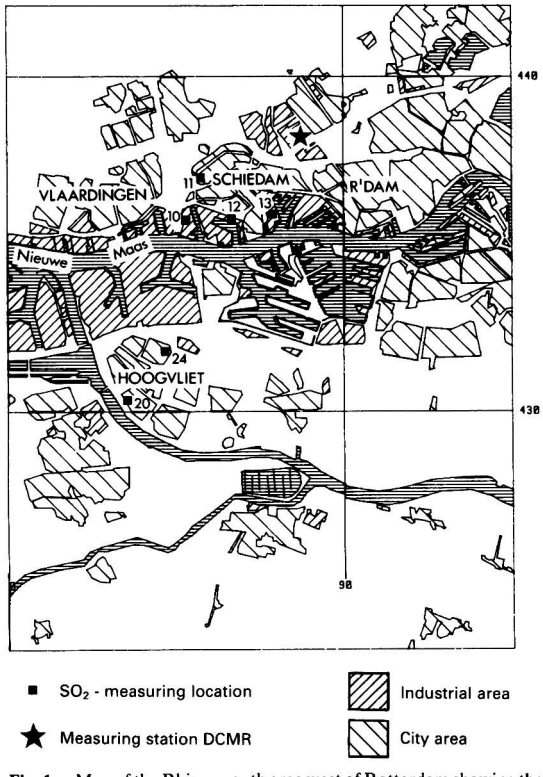

Fig. 1. Map of the Rhine-mouth area west of Rotterdam showing the automatic SO_2 sampling and measuring locations

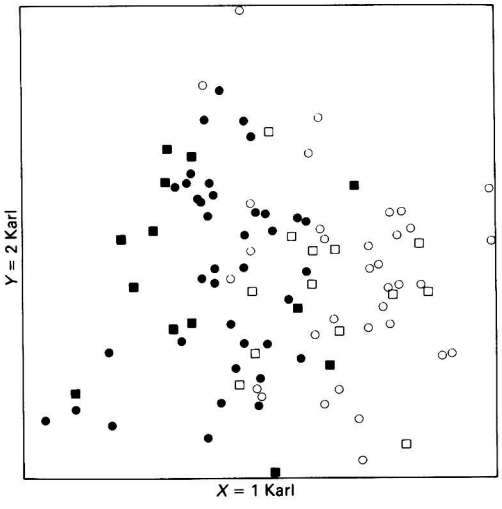

Fig. 2. Karhunen-Loève projection of logarithmised features: ○, non-complaint hours (training set); ●, complaint hours (training set); □, non-complaint hours (test set); ■, complaint hours (test set)

found (at 13.00 and 14.00 h). Hereafter the trace moves out of the complaint area and the observation period ends with non-complaints in the non-complaint region. The nearest neighbour technique operates well here.

In order to demonstrate the failure of the same learning machine, a test set of 24 successive hours from July 9th, 13.00 h, to July 10th, 12.00 h, 1982 is applied in the same way as that of May 17th (Fig. 4). Here all hours linger in the same area, denoted by a moderate probability of complaints. Sometimes a complaint is actually made, but prediction is virtually impossible. Application of the nearest neighbour technique on these 24 hours does not yield a good classification here.

The results so far show the need for a more probabilistic descriptor, which will be given in the next section.

Bayes Statistics

Probablistic Description of Complaint Situations

From an observation of the Karhunen - Loève projections of pattern vectors denoting complaint and non-complaint hours, it is seen that no sharp distinction can be made between a complaint area and a non-complaint area. There exists an in-between area with a non-zero probability for each of the two categories.

The assumption can be made that on a line connecting the centres of gravity of the non-complaint area and the complaint area the probability for a complaint increases from about 0 to about 1 in a sigmoidal way.

Such a relationship is also observed when a test panel is exposed to an increasing concentration of unpleasant smelling (noxious) air. The number of persons from the panel indicating that they could smell something bad increases gradually with increasing logarithm of the concentration of pollutant.

In order to describe these phenomena, a Bayesian statistical approach is chosen.[3] In accordance with such an approach, a probability is attributed to each position in the pattern space given the chance of a complaint situation. This probability function is constructed from knowledge derived from a training set with a statistical amount of complaint and non-complaint situations. The reliability of this function depends critically on the availability of a representative training set.

For a two-category model, as encountered here, the probability for x (x being a member of one category out of n categories) is given by

$$p[x] = p[x|a(1)]*p[a(1)] + p[x|a(2)]*p[a(2)] + \ldots + p[x|a(n)]*p[a(n)] \quad \ldots \quad (2)$$

Table 3. Classification according to five nearest neighbours for the training set consisting of 35 non-complaint hours and 38 complaint hours (%)

	Predicted category	
Input category	Non-complaint	Complaint
Non-complaints	77	23
Complaints	13	87

Table 4. Classification according to five nearest neighbours for a test set consisting of 13 non-complaint hours and 13 complaint hours (%)

	Predicted category	
Input category	Non-complaint	Complaint
Non-complaints	69	31
Complaints	15	85

where $a(i)$ are categories with $i = 1,2,3, \ldots, n$. $p[a(i)]$ equals the *a priori* probability for the ensemble $a(i)$ and $p[x|a(i)]$, the conditional probability for encountering x when ensemble $a(i)$ is given.

In Bayes statistics the question is reversed: if an event x is encountered, then what is the probability that this event belongs to the ensemble $a(i)$?, or, find $p[a(i)|x]$. The probability that event x belongs to ensemble $a(i)$ is then compared to the probability that event x belongs to all other ensembles $a(j)$.

For a two-category model, this comparison results in a decision function

$$s(x) = \frac{p(1)\,L(1)\,p(x|X_1)}{p(2)\,L(2)\,p(x|X_2)} \quad \ldots \quad (3)$$

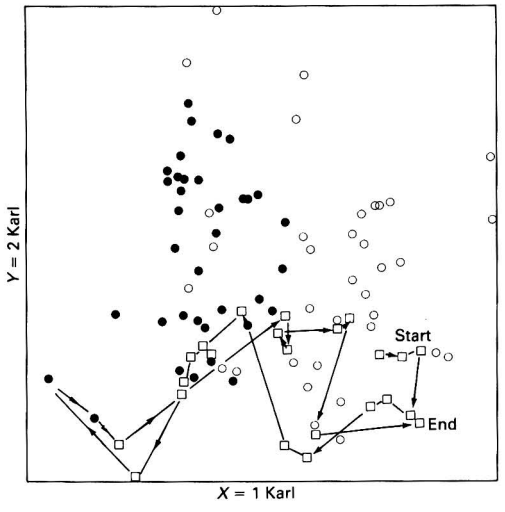

Fig. 3. Evolution of a complaint situation in time for May 17th 1982, followed from 01.00 h until 24.00 h, projected on the training set of Fig. 2. □, Hours of May 17th; □, start (01.00 h); □, end (24.00 h)

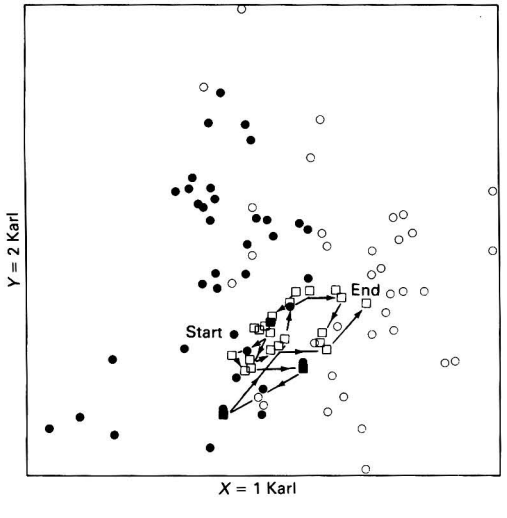

Fig. 4. Evolution of a complaint situation in time for July 9th and 10th 1982, followed from 13.00 h until 12.00 July 10th. □, Hours of July; □, start (9th, 13.00 h); □, end (10th, 12.00 h)

where $p(x|X_1)$ represents the probability function for event x belonging to category 1, $p(x|X_2)$ the same function for category 2, $p(1)$ equals the *a priori* probability of category 1 and $p(2)$ that of category 2. $L(1)$ represents the loss function for category 1, a number between 0 and 1 describing the penalty on a misclassification in the other category; the same applies to $L(2)$.

The classification of an unknown pattern x in category 1 occurs when $s>1$; otherwise it is classified in category 2. This decision function is only valid if a one-dimensional probability function $p(x)$ can be calculated from all features in the pattern space.

The Bayes classifier for the ARTHUR computer package, however, does not allow this calculation. Instead, the probability function is calculated for each individual feature and all these probability functions are combined afterwards. Thus, the probability for the occurrence of x in category k based on the knowledge of the value for feature j equals

$$p_j(X_{jk}|x_j) = \frac{p(k)\,L(k)\,p(x_j|X_{jk})}{\sum_{k=1}^{K} p(k)\,L(k)\,p(x_j|X_{jk})} \quad .. \quad (4)$$

in which K represents the number of categories (here 2). The probability distribution function for feature j is estimated from the histogram of this feature, derived from the training set patterns. For the combination of the individual distribution functions per feature, the ARTHUR package gives two alternatives:

$$P_{\text{total}}(X_k|x) = C * \exp\{\sum_{j=1}^{J} \ln[p_j(X_{jk}|x_j)]\} \quad .. \quad (5)$$

or, alternatively

$$P_{\text{total}}(X_k|x) = C(\alpha) * \sum_{j=1}^{J} [p_j(X_{jk}|x_j)]^{\alpha} \quad .. \quad (6)$$

where C and $C(\alpha)$ represent normalisation constants.

Implementation of the Bayesian Classifier for our Problem

The 25 class resolution histograms of all features except the wind direction had a Gaussian distribution function. The resolution of the distribution over 25 classes proved experimentally to be sufficiently accurate.

The selection of a loss function encounters a basic difficulty; because no knowledge is available, each selection is a subjective one. In such a situation the maximum likelihood classification is chosen, *i.e.*, an equal loss factor, valued 1, for all categories. Another assumption made is that all new, unknown patterns of the test set are distributed according to the same distribution function as those from the *a priori* known patterns of the training set.

Selection of test sets from the available data

Three test sets were selected: test set one consists of 20 complaint hours and 19 non-complaint hours in 1982; test set two consists of 18 complaint hours and 18 non-complaint hours in 1981; test set three consists of all complaint hours in 1982 as far as no missing feature values are met. It contains 332 pattern vectors, but not all of these complaint hours satisfy the conditions mentioned in the introduction.

The training set consists of three categories, described as follows: category one with high values for NO_x, low for SO_2 and low wind speed, yielding complaints from the population; category two, having high values for SO_2 and a wide distribution for the values of NO_x, wind coming from the industrial area, yielding complaints from the population; category three, no complaints, features show widely diverging values.

The features for the description of the training set are NO, NO_2, SO_2 measured at four different locations, the wind velocity and the wind direction (0 or 1) summed over a period of 5 h.

In order to optimise the predicting power of the learning machine, the following *a priori* probabilities (non-normalised) were chosen: $p(1) = 0.1$, $p(2) = 0.3$ and $p(3) = 1.0$. With these probabilities and the histograms for all features, the individual feature probabilities were combined according to equation (5). In Table 5 the results of the classification of the combined classes 1 and 2 (denoted "complaint") and those of class 3 (denoted "non-complaint") are presented for the training set and the test sets one and two.

From Table 5 it can be seen that the complaint hours are well classified (80–81% correct), which differs only to a minor extent from that of the training set (84%). However, a test with all the 332 complaint hours of 1982 (without missing features) reveals that only 65% of all hours are classified correctly. This may be interpreted as decisions resulting from an intermediate area in the pattern space, where the probability for classification as complaint hour is almost equal to that for non-complaint hours. This interpretation is illustrated in Fig. 5, where the probability that the patterns of test set three belong to category 2, calculated by the Bayes classifier according to equation (5), is plotted. It is seen that this classifier behaves as a yes/no decision function, because all intermediate probabilities have a population of practically zero. This observation is in conflict with the expectation of an intermediate area where intermediate probabilities should be denoted.

In a search for a better representation of this area, the feature probability functions were combined according to equation (6), with application of an optimised value for α and $p(i)$ (i = 1, 2 and 3).

The optimisation, following an iterative procedure, was aimed for a maximum amount of correctly classified training set patterns in all categories, and resulted in the parameter values given in Table 6. From a comparison of the classification results, presented in Tables 5 and 6, it is seen that the actual classification hardly shows any difference. Test set three, comprising all complaint hours from 1982, is here only 64% correctly classified. However, the probabilities for category 2 of these complaints (Fig. 6) behave differently. Here the intermediate region is well populated and therefore this Bayesian classifier is not a simple yes/no one. For the decision function

$$s(x) = \frac{\text{probability}(x) \text{ for class 2}}{\text{probability}(x) \text{ for class 3}}$$

operating on all complaint hours of 1982, Fig. 7 is found, which on integration yields Fig. 8.

The shape of this function corresponds well with a graphical representation of the experiment, mentioned before, where observations were made of a test panel that was exposed to air

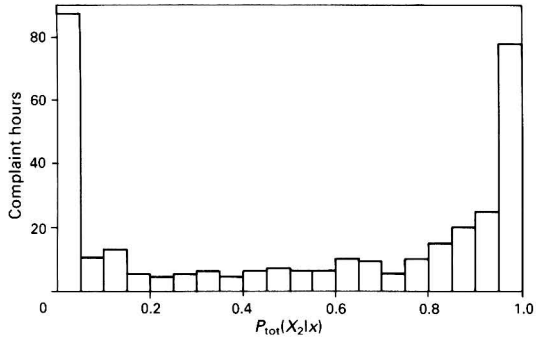

Fig. 5. Histogram of complaint hours of class 2 as a function of the Bayesian classifier equation (5)

with an increasing concentration of a noxious smelling odour. Here the number of observations on bad odour was plotted as a function of the logarithm of the concentration.

The correspondence of these two functions suggests a relationship between the probability function $s(x)$ and the burden felt by the population in the area, resulting in an increasing number of complaints. This will be elaborated under Quantification of the Burden of Air Pollution.

Quantification of the Burden of Air Pollution

One of the possibilities offered by pattern recognition is the ability to estimate unmeasurable properties of an object by the observation of measurements. Here the measurements are concentrations of chemical constituents of air and meterological conditions, as a function of time and location. From this base one aims for a yardstick for the amount of burden that is felt by the inhabitants of the area, which cannot be "measured" directly.

In a search for the relationship between the amount of burden and the value of the decision function $s(x)$, the interval between the minimum and maximum values of $s(x)$ encountered in practice was subdivided into nine intervals. The spacing of these intervals was chosen such that about equal amounts of complaint hours are found in each of the intervals (Table 7).

The measurements describing each of the individual intervals are fed as separate categories to the ARTHUR computer program. Histograms for the various features are constructed and the weights for category separations are calculated. From the inspection of the results of these calculations it is seen that the velocity and the direction of the wind are mainly responsible for elevated SO_2 concentrations, provided that there is high industrial activity, and for high NO and NO_2 concentrations, resulting from automotive traffic. A high wind velocity produces a high mixing and dilution of these components, resulting in a limited burden on the population and a low value for the decision function $s(x)$.

The direction of the wind becomes more important with a decrease in wind velocity: a prolongated wind direction from the industrial area results in an enhanced concentration of, *e.g.*, SO_2 and thus an increased burden; $s(x)$ also increases here.

A further decrease in wind velocity is accompanied by enhanced concentrations of NO and NO_2. In combination with low industrial activity, SO_2 concentrations will remain low and hourly reports according to category 1 of the training set will be encountered.

However, a low wind velocity generally means that the direction of the wind will be undefined. This type of weather, in combination with high industrial activity, means that the concentrations of SO_2 increase at all locations. Here discrimination based on the location of the measurement becomes less significant. In combination with the low mixing and dilution, the decision function becomes very high, together with the burden felt.

Because of this, one may conclude that $s(x)$ increases with an increase in SO_2 concentration, which itself serves as a tracer for all kinds of noxious smelling substances produced by industrial activities. Thus $s(x)$ is interpreted as a yardstick for the burden felt by the population. In order to elaborate on this point of view, a search is made for the correlation between $s(x)$ and the number of filed complaints.

For every hour of 1982, as far as no missing feature values were encountered, $s(x)$ is calculated and classified in one of the classes of Table 7. It is seen in this table that complaints were filed in 332 hours out of the 5511 tested hours, yielding a

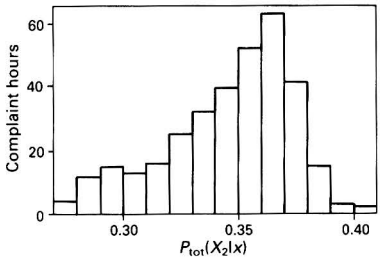

Fig. 6. Histogram of complaint hours of class 2 as a function of the Bayesian classifier equation (6)

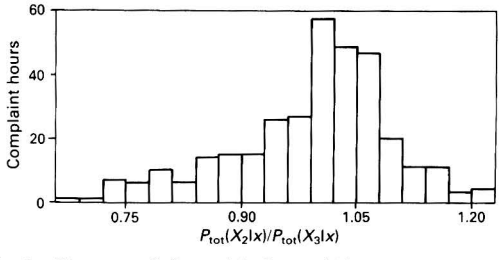

Fig. 7. Histogram of all complaint hours of 1982 as a function of the probability ratio for class 2 over class 3

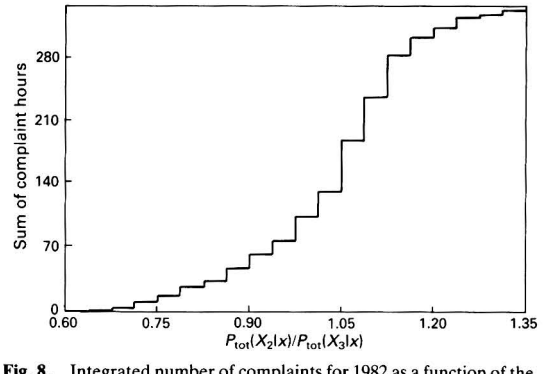

Fig. 8. Integrated number of complaints for 1982 as a function of the probability ratio for class 2 over class 3

Table 5. Classification with a Bayesian classifier, according to equation (5). $p(1) = 0.1$; $p(2) = 0.3$; $p(3) = 1.0$

		Predicted category	
Set	Input category	Non-complaint	Complaint
Training set ..	Non-complaint	69	31
	Complaint	16	84
Test set one ..	Non-complaint	53	47
	Complaint	20	80
Test set two ..	Non-complaint	61	39
	Complaint	19	81

Table 6. Classification with a Bayesian classifier according to equation (6). $\alpha = 0.2$; $p(1) = 3.27$; $p(2) = 3.29$; $p(3) = 3.44$

		Predicted category	
Set	Input category	Non-complaint	Complaint
Training set ..	Non-complaint	73	27
	Complaint	15	85
Test set one ..	Non-complaint	53	47
	Complaint	20	80
Test set two ..	Non-complaint	61	39
	Complaint	22	78

Table 7. Boundary values between successive $s(x)$ classes, population of classes (hours and complaint hours), % complaint hours and number of complaints per 100 h (*i.e.*, burden on inhabitants of the area) for the year 1982

Class value, i	Boundary between $s(x)\,i$ and $i-1$	Total number of hours in i	Number of Complaint hours in i	% Complaint hours in i	Average number of complaints per complaint hour	Number of complaints per 100 h
1	—	1808	35	1.9	1.11	2.1
2	0.849	913	37	4.1	1.08	4.4
3	0.918	768	36	4.7	1.42	6.7
4	0.965	542	40	7.4	1.14	8.3
5	0.999	303	31	10.2	1.67	17.3
6	1.015	276	34	12.3	1.53	18.8
7	1.031	402	38	9.5	1.82	17.4
8	1.061	249	36	14.5	1.46	21.3
9	1.087	250	45	18.0	1.67	31.6
Total (average):		5511	477	(6.0)	(1.43)	(8.6)

total of 477 complaints. The distribution of these hours over the various classes is such that the number of complaints increases with a higher class number. The average number of complaints per complaint hour does not vary in a systematic fashion over the classes (Table 7).

Column 7 in Table 7 is constructed by multiplication of the number of complaint hours per class with the average number of complaints per hour and divided by the total number of hours per class. Column 7 of Table 7 gives an indication of the burden felt by the inhabitants of the area, as a function of the class number, *i.e.*, the value of the decision function. It is seen that in classes 1–3, the number of complaints per 100 h gradually increases. In classes 4 and 5 this number is almost doubled. Classes 5, 6 and 7 have almost the same value whereas in classes 8 and 9 a jump in the value of this number is again noticed.

This table indicates that there is a relationship between the weight of the burden and the amount of complaints, although the number of hours with more than one complaint does not change significantly. The relative number of complaint hours does increase.

It should be noted that for the compilation of Table 7, all hours with no missing feature values are taken into account. This means that hours between 02.00 and 08.00 h are also included. From the distribution of complaints over the day (see Appendix), it is known that in this period almost no complaints are made. When a correction is made for this observation, the number of complaints per 100 h should be increased by almost 30%.

Conclusions

The load of the burden of noxious air on the population has been characterised by concentrations of NO, NO_2 and SO_2. These concentrations depend on wind direction and wind velocity. In Schiedam, a city in the Rhine-mouth area, a wind direction from the south west will give enhanced concentrations at locations 10 and 11 (Fig. 1). A low wind velocity gives rise to a low mixing of air and a cumulation of all eventual noxious constituents. A very low wind speed results in a varying wind direction and a wider area over which noxious constituents may be spread.

In feature space, there does not exist a sharp boundary between hours with complaints and hours with no complaints. Instead a probablistic description should be handled.

Bayes statistics, based on past experience, offer the possibility of constructing such a probablistic description. A decision function can be made that is based on the ratio of the probability for complaints and the probability for non-complaints. The numerical result of this decision function can be regarded as a measure of the seriousness of burden on the population, which in turn is reflected by the complaints filed.

According to this method, it may be possible in practice to estimate the number of complaints at a given time, once the necessary features are obtained: the burden, "measured" by this method, is an indication of this. When the time-dependent effects of the burden on the population have been investigated more thoroughly, this method may not only help to predict the burden but, more importantly, help to prevent it.

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APPENDIX

Some Statistical Information on the Observations During 1982

Total number of hours: 8760. Hours with one or more complaints: 499. Total number of complaints: 770.

The frequency distribution of complaints is indicated in Table 8 and histograms of the number of complaints are shown in Figs. 9 and 10.

Table 8. Frequency distribution of complaints in complaint hours

Number of complaints/h	Number of hours
1	381
2	73
3	19
4	6
5	8
6	2
7	1
8	5
9	0
10	1
>10	3

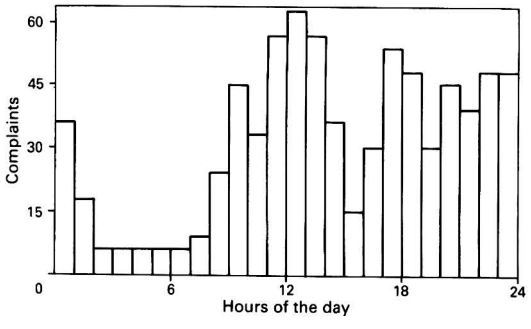

Fig. 9. Histogram of the number of complaints as a function of the hour of the day

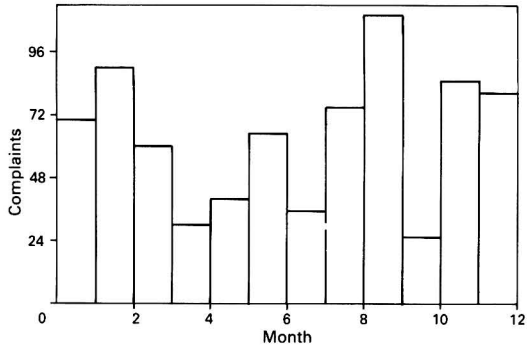

Fig. 10. Histogram of the number of complaints as a function of the month of the year

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Flow Cell Studies With Immobilised Reagents for the Development of an Optical Fibre Sulphide Sensor

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Several reagent systems, such as 2,6-dichlorophenolindophenol, phenanthroline complexes and dithiofluorescein complexes, have been immobilised on polymeric solid supports and their suitability as interphases in optical fibre sensors for sulphide ions investigated. These studies were carried out in a flow cell arrangement. The reflectance of the reagent phase was measured using optical fibres and was found to be related to the concentration of the sulphide ions in the solution. The dynamic range for sulphide ion determination varied with the reagents. The reagent phases prepared from 2,6-dichlorophenolindophenol and from the complex formed by dithiofluorescein with *o*-hydroxymercuribenzoic acid could be regenerated and are considered suitable for the development of an optical fibre sensor for sulphide ions.

Keywords: *Sulphide determination; optical fibre sensor; immobilised reagent systems; flow cell*

The advantages of chemical sensors based on optical fibres have been widely recognised, and consequently the development of sensors of this type has been pursued with intense interest. These sensors are based on the change in the optical characteristics of a reagent phase incorporated on the tip of an optical fibre, through which the change is detected. The reagent phase usually consists of a chemically specific reagent immobilised on a polymeric solid support.

A variety of optical fibre chemical sensors have already been reported. Optical sensors for pH,[1,2] moisture[3] and ammonia[4] have been devised, based on a change in the absorbance or reflectance of a reagent phase in the presence of the analyte. Fluorescence sensors for pH,[5,6] some metal ions,[7-9] halide ions,[10] oxygen,[11] carbon dioxide,[12] glucose[13] and haloethanes[14] have also been constructed using optical fibres.

This paper presents preliminary studies on the development of an optical fibre sensor for sulphide ions. Several reagent phases have been immobilised on a solid support and their reaction with small amounts of sulphide ions was investigated. The suitability of the reagent phases in an optical sensor have been evaluated. Based on these results, an optical fibre probe for sensing sulphide ions is being explored.[15] This further study will also demonstrate the applicability of the reagent systems in optosensing as detectors for sulphide ions in a flow system similar to that described by Růžička, and Hansen.[16]

The development of an optical fibre sulphide ion sensor could provide a simple and rapid method for routine environmental measurements. Small amounts of sulphide ions in effluents and atmospheres produce effects toxic to living organisms, together with corrosion in metallic and concrete structures. Although its characteristic odour could be a means of detection of sulphide, this is not reliable because the nose is desensitised by high concentrations of sulphide ions. An optical fibre sensor provides an alternative to the electrochemical sensors now in use as it is capable of being used for remote monitoring and is not susceptible to electromagnetic interference.

Experimental

Instrumentation

The instrumentation (Fig. 1) used in this study has been adapted from a system previously described for pH measurement with an optical fibre.[1] Optical radiation was supplied by a quartz - halogen lamp (12 V, 100 W) and modulated by an optical chopper (Bentham 218) set at a frequency of 360 Hz.

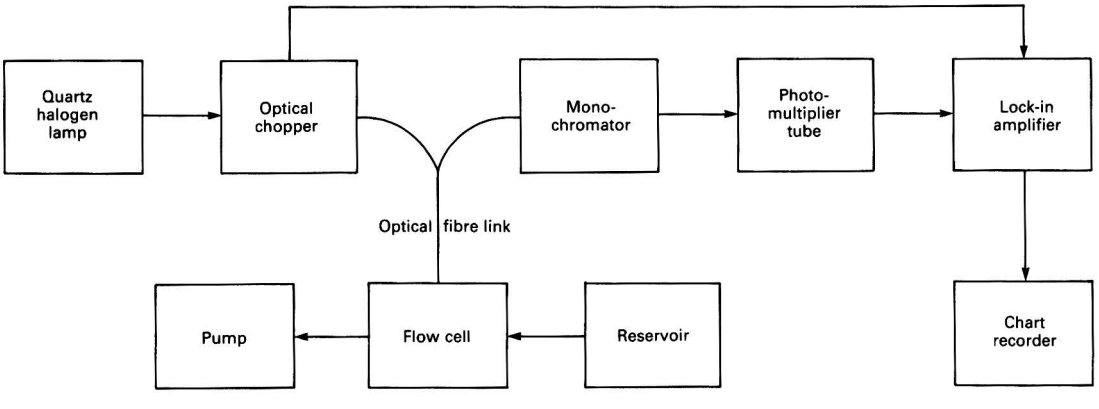

Fig. 1. Schematic diagram of the instrumentation system used for reflectance measurements

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The reagent phase, contained in a flow cell, was irradiated through one branch of a bifurcated fibre system, constructed from a 16-polymer optical fibre bundle (Optronics). Light reflected from the reagent phase was collected and guided to a monochromator (ISA Instrument H-1061) by the other branch of the optical fibre system. Measurement of the reflected radiation was carried out with a photomultiplier (Hamamatsu R446), a current amplifier (Bentham 286) and a lock-in amplifier (Bentham 223). The reflectance spectra and the response graphs were recorded on a chart recorder (Servoscribe RES11).

The flow cell (Fig. 2) was fabricated from a Perspex block and featured a bore (2.2 mm in diameter) perpendicular to the axis of a cylindrical compartment (6 mm long and 3 mm in diameter). A fine wire mesh was placed at one end of the compartment to block the reagent phase, which was loaded into the flow cell with the aid of a peristaltic pump (Watson-Marlow 21) and packed in such a manner that it completely surrounded the sensing end of the fibre. The reagent phase was continuously flooded with either water, a buffer or an analyte solution during reflectance measurements. Bubbles would disrupt the arrangement of the microspheres in the packing and alter the reflectance; consequently, care was exercised to exclude them from the flow.

Reagents

Several spectrophotometric reagents for sulphide ion determination were used in the study. Solutions of phenolindo-2,6-dichlorophenol (2,6-dichlorophenolindophenol, DIP), tris(1,10-phenanthroline)iron(III) sulphate and bis(2,9-dimethyl-1,10-phenanthroline)copper(II) sulphate were prepared from chemicals purchased from BDH Chemicals. Solutions of the complex of dithiofluorescein (Fluorochem) with silver nitrate (BDH Chemicals) and with *o*-hydroxymercuribenzoic acid (Koch-Light) were prepared according to the method of Wronski.[17,18]

Silica gel and some porous organic polymers, all obtained from BDH Chemicals, were investigated as possible supports for the immobilisation of the reagents. The organic materials used were Amberlite XAD-2, XAD-4 and XAD-7. Whereas XAD-2 and XAD-4 are cross-linked copolymers of styrene and divinylbenzene, XAD-7 is a cross-linked polymer of methyl methacrylate.

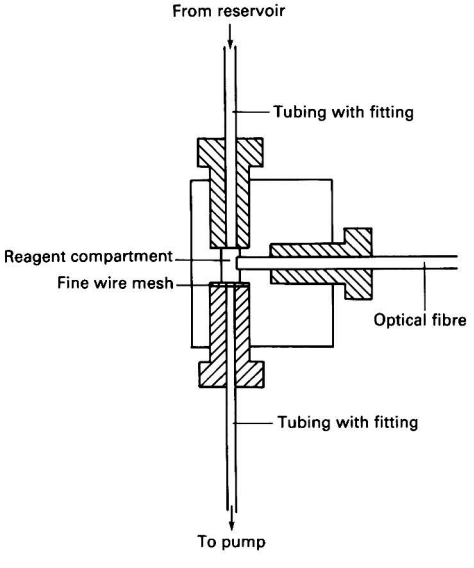

Fig. 2. Cross-section of the flow cell assembly

Immobilisation Procedure

Immobilisation consisted in soaking the immobilising agent (0.5–1.0 g) in 25 ml of a solution of the reagent (0.05–1.0%) for 2–4 h. At the end of this period, the solid phase, which contained the adsorbed reagent, was thoroughly washed with water until the washings were colourless. The optimum conditions (*i.e.*, solution concentration, amount of adsorbent and immobilisation period) for each reagent were determined by spectrophotometric analysis of the liquid phase in the system.

For the organic immobilising agents, the microporous polymer beads were washed successively with acid, water and methanol. The non-polar polymers, XAD-2 and XAD-4, had to be hydrated before they could be used in aqueous solutions. This was achieved by soaking the polymer first in methanol and then in water.

Reflectance Measurements

The reflectance spectra of each reagent phase were recorded before and after its reaction with sulphide ions. The wavelength of maximum divergence between the two spectra was identified and used in subsequent reflectance measurements.

Results and Discussion

2,6-Dichlorophenolindophenol (DIP)

Sulphide ions decolourise DIP, reducing it from the blue azoquinoid form into the leuco form. The reagent was best immobilised on Amberlite XAD-2 microspheres, which acquired a violet colour as a result of the adsorption of the dye.

The colour was discharged in the presence of sulphide ions and recovered through oxidation with oxygen. The above reactions of the reagent in the immobilised state were observed to be faster than those in solution. The reflectance spectra recorded for the immobilised reagent before and after reaction with sulphide ions are illustrated in Fig. 3.

The response obtained from reflectance measurements at 598 nm was a function of the concentration of the sulphide ions. The relative reflectance of the reagent phase increased linearly with the logarithm of the concentration of sulphide ions in the range 8–20 mmol l^{-1} (Fig. 4). The reaction rate was affected by the pH of the analyte and was greatest at pH 8. The response time varied between 2 and 25 min, depending on the sulphide ion concentration. Reflectance signals were therefore recorded after 2 min of reaction.

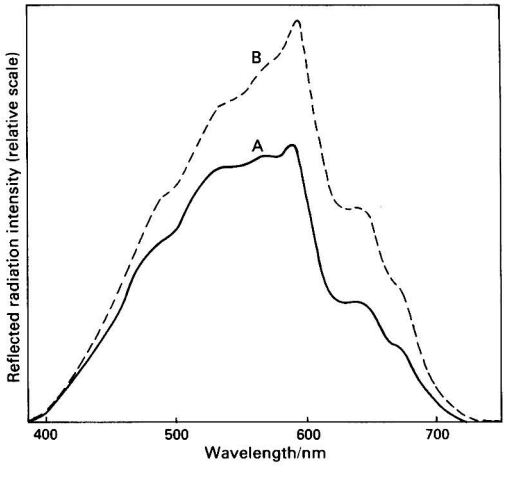

Figure 3. Reflectance spectra obtained from DIP immobilised on XAD-2 (A) before and (B) after reaction with sulphide

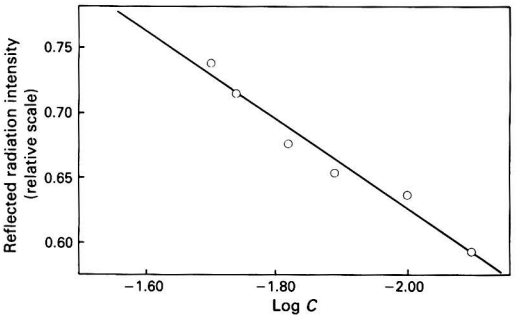

Fig. 4. Semi-logarithmic graph of the reflectance of immobilised DIP with different concentrations of sulphide (mol l^{-1})

Phenanthroline Complexes

Tris(1,10-phenanthroline)iron(III) is converted into a bright red complex ion through the reduction by the sulphide ion of the iron atom in the coordination sphere. This complex could be suitably held on the surface of Amberlite XAD-7 microspheres. The resulting immobilised reagent was sensitive to small amounts of sulphide ions and showed a 25% reduction of its reflectance at 550 nm in a solution containing 0.16 mmol l^{-1} of sulphide. However, it was noted to be unstable, and was transformed into its reduced form during storage, particularly in the presence of light. Similar observations have been reported in solution studies.[19]

Bis(2,9-dimethyl-1,10-phenanthroline)copper(II) reacts with sulphide ions and produces a bright orange complex ion as a result of the reduction of the copper ion. This coordination compound could be adsorbed on silica gel and on Amberlite XAD-7. A weak luminescence, which has been reported for the complex in non-aqueous solutions,[20] was also detected in the reagent immobilised on silica gel. This emission interfered with the reflectance measurements. On the other hand, the copper complex held on XAD-7 showed a marked change in its reflectance spectra in the presence of sulphide ions. The response at 485 nm was rapid and reproducible. The reflectance signals read after 33 s of reaction showed a linear dependence on the logarithm of the concentration of the sulphide ion in the range 0.13–0.50 mmol l^{-1}. It was noted that the reflectance approached the same equilibrium value for all the concentrations of sulphide ion used in the study. The equilibrium was found to be insensitive to the pH of the system. Regeneration of the reagent phase through oxidation with oxygen gas or with hydrogen peroxide was attempted, but proved unsuccessful. This irreversibility is also observed in solutions of the reagent and has been attributed to the stabilisation of the copper(I) complex by the steric hindrance of the methyl substituents.[21]

Dithiofluorescein Complexes

The coordination compounds formed by the reaction of dithiofluorescein (DTF) with silver nitrate and with *o*-hydroxymercuribenzoic acid (HMB) were observed to react with sulphide ions, releasing the blue DTF dye. Immobilisation of these reagents from their aqueous solutions resulted in a solid phase that was not responsive to the presence of sulphide ions. A sulphide-sensitive reagent phase was produced through a preliminary immobilisation of DTF on a suitable matrix (XAD-2) and a subsequent reaction of the adsorbed dye with silver nitrate or HMB. A plausible explanation for the difference in the properties of the reagent phases resulting from a direct immobilisation and a stepwise formation is that there is a difference in the orientation of the adsorbed reagent molecule on the surface of the supporting solid and in the degree of accessibility of the metal atom for reaction with the sulphide ion.

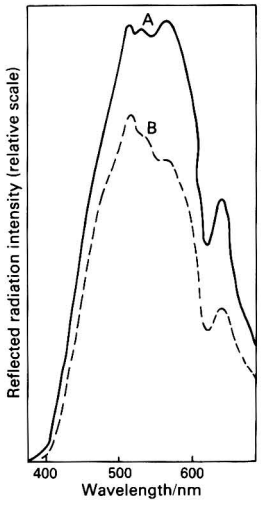

Fig. 5. Reflectance spectra obtained from Ag - DTF immobilised on XAD-2 (A) before and (B) after reaction with sulphide

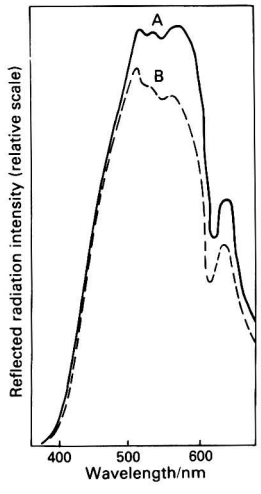

Fig. 6. Reflectance spectra obtained from HMB - DTF immobilised on XAD-2 (A) before and (B) after reaction with sulphide

A distinct change in the reflectance spectra of the reagent phases occurred on their reaction with sulphide ions, the greatest change occurring at 590 nm (Figs. 5 and 6). The response obtained at 590 nm varied linearly with the logarithm of the concentration of sulphide ions in the range 0.063–0.63 mmol l^{-1} for the silver complex and 0.31–2.5 mmol l^{-1} for the HMB complex (Fig. 7). The response was rapid, reaching a minimum after 20 s of reaction. A constant value for the reflectance of the reacted reagent was not observed because of the photo-instability of the resulting DTF.[22]

The reagent phases were regenerated by complexing the liberated DTF with a solution of silver nitrate or of HMB passing through the flow cell. The recovery of the original phase was possible for the immobilised HMB complex, but not for the immobilised silver complex. Silver sulphide formed

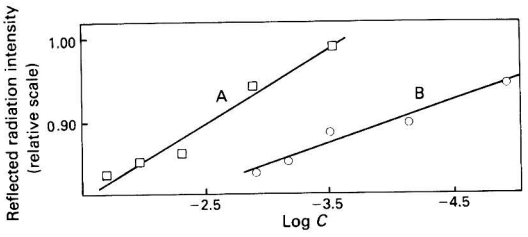

Fig. 7. Semi-logarithmic graphs of the reflectance of (A) Ag - DTF and (B) HMB - DTF immobilised on XAD-2 with different concentrations of sulphide (mol l^{-1})

during the reaction of the immobilised silver complex with sulphide ions was found to be deposited on the surface of the polymer support and prevented the recovery of the colourless reagent phase.

Conclusions

The results of this study demonstrate the suitability of the immobilised reagents for measuring small amounts of sulphide ions. In general, the reactions are observed to be faster than those occurring in solution, and the responses are found to be reproducible. The reflectance of the reagent phases studied varies linearly with the logarithm of the concentration of sulphide over certain ranges. The immobilised DIP and DTF - HMB complexes are renewable and could therefore be used in optical fibre probes for sulphide ions. These reagent phases can also be used in flow-through detectors for sulphide ions in a manner similar to that described by Růžička, and Hansen.[16] The immobilised copper - phenanthroline complex and the silver - DIF complex cannot be regenerated, but would nevertheless be useful in disposable sensors and alarm systems based on optical fibres.

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Collaborative Studies of Methods for the Detection of Residues of Monensin in Chicken Tissues

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Some published methods for the detection of residues of monensin in chicken tissues were examined. In some instances the limits of detection claimed by the authors could not be achieved. A method was evaluated in which the monensin was extracted from the tissues with methanol, partitioned into carbon tetrachloride and then purified on a silica gel column. Monensin was determined in the eluate from this column by TLC. Alternative procedures for detecting the monensin on the TLC plate by bioautography or by formation of a fluorescent derivative are given.

Keywords: *Monensin residues; chicken tissues; collaborative studies; thin-layer chromatography*

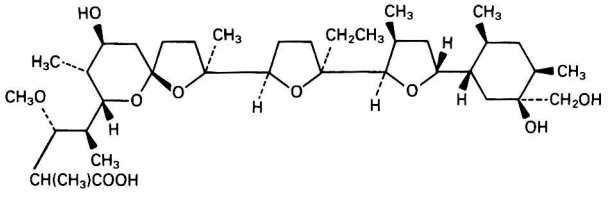
The Analytical Methods Committee has received and has approved for publication the following report from its Veterinary Residues in Fresh Meat Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Dr. J. F. C. Tyler (Chairman, until April 1984), Dr. N. T. Crosby (Chairman, from May 1984), Mr. J. B. Aldred, Mr. A. Anderson, Mr. K. J. Briant (from May 1984), Mr. P. M. Brown, Mr. J. Ganley, Mr. H. L. Hatfield, Mr. A. Hobson-Frohock, Mr. A. F. Lott, Mr. A. F. Machin (until April 1984), Mr. M. P. Quick (from September 1984), Mr. R. Ryden, Dr. G. Shearer and Mr. G. M. Telling, with Mr. J. J. Wilson as Secretary.

Introduction

Monensin (Chemical Registry No. 17090-79-8) is a polyether antibiotic produced by *Streptomyces cinnamonensis*. It is added in the form of the sodium salt to broiler chicken feeds as a coccidiostat at levels of 100–120 mg kg^{-1} until 3 d before slaughter. It is also used as a growth promoter in cattle feeds. Monensin ($C_{36}H_{62}O_{11}$) has a relative molecular mass of 670.9 and the structure shown below.



The compound itself is non-volatile and does not absorb in the UV - visible region. This restricts the number of chemical techniques that are available for its determination in animal feeding stuffs. The low levels likely to be present in tissues further exacerbate the problem.

Kline *et al.*[1] proposed a microbiological assay for monensin in feeds using *Bacillus subtilis*, whereas Golab *et al.*[2] described a spectrophotometric procedure based on the reaction of monensin with vanillin (4-hydroxy-3-methoxybenzaldehyde). An HPLC method using a refractive index detector has been reported by Macy and Loh,[3] but this is only applicable to pre-mixes.

Similar techniques have been utilised for the determination of monensin in animal tissues. Donoho and Kline[4] described a semi-quantitative thin-layer bioautographic method, which was later modified by Okada *et al.*[5] Tihova and Peneva[6] also used TLC to separate monensin from co-extractives, but employed *p*-anisaldehyde (4-methoxybenzaldehyde) as a detection reagent in place of bioautography as the end determination step. Other medicinal additives such as lasalocid, narasin and salinomycin, which are very similar in chemical structure to monensin, may also be present and would not be separated by the above methods. However, whilst this work was in progress, Owles[7] reported a qualitative test for the separation of these antibiotics in feeds and pre-mixes using a TLC system.

The Sub-Committee was charged with the task of developing and evaluating a method for the detection and determination of monensin residues in chicken tissue at a level of 0.1 mg kg^{-1} or below. The Sub-Committee began by examining the method of Okada *et al.*[5], which uses a thin-layer bioautographic technique at the determinative stage. The authors claimed that recoveries of monensin from fortified tissues were 92.9% from fat, 86.0% from liver and 104.4% from muscle tissues. The detection limit achieved was 0.01 mg kg^{-1} in fat and 0.0125 mg kg^{-1} in other tissues. No detectable residues of monensin were found in the fat of animals 48 h or more after withdrawal from the feed, or in other tissues 24 h or more after withdrawal.

In later work, the method of Tihova and Peneva[6] was evaluated. This also used TLC to separate monensin from co-extractives followed by detection with *p*-anisaldehyde reagent to form a fluorescent derivative. This method has the advantage that it only uses equipment and experience normally found in chemical laboratories and does not require microbiological techniques. The authors claimed a better sensitivity (0.003 mg kg^{-1}) than Okada *et al.*[5] and a mean recovery of 97% from sheep and pig tissues. A simple solvent partition stage was recommended for the removal of co-extractives, in contrast to the column chromatography system used by Okada *et al.*[5]

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Experimental

Investigations were carried out using samples of chicken tissues kindly supplied by the Lilly Research Centre. These were obtained from animals fed on diets known to be free from monensin. The feeding of chickens with monensin at recommended levels in the diet would produce residual levels in tissues that are likely to be too low to be useful for the evaluation of an analytical method. On the other hand, the birds might not accept feeds containing significantly higher levels of monensin than those recommended. Hence, it was decided to work only on monensin-free tissues to which known amounts of monensin had been added in the laboratory.

Table 1. Recovery of monensin residues from fortified tissue. Bioautographic method

Laboratory	Tissue	Monensin added/mg kg^{-1}	Estimated recovery, %
1	Muscle	0.05	67, 67
		0.1	67, 53
	Liver	0.05	94, 76
		0.1	89
2	Muscle	0.0125	72, 72, 65
		0.1	40, 80, 70
3	Muscle	0.0125	0, 0, 57, 81, 33, 53
		0.025	50, 64, 81, 64
		0.1	84, 75, 106
4	Muscle	0.0125	0, 0, 0, 0, 0
		0.025	74, 73, 63, 73
		0.05	82, 94, 68, 62, 77, 62, 67, 53, 62
		0.1	54, 55, 63, 64, 64
		0.2	62, 70, 58
	Liver	0.05	108, 109, 118, 99, 89
5	Muscle	0.1	117, 150, 140
		0.1	125, 112
	Liver	0.1	50, 60
6	Muscle	0.017	110
		0.033	78
		0.05	88, 88, 89, 93
		0.066	79
	Liver	0.05	87, 77, 72, 72

Evaluation of the Method of Okada *et al.*[5]

Preliminary studies of the bioautographic procedure established that levels of monensin down to 0.1 mg kg^{-1} could be detected without much difficulty. At lower levels, difficulties were encountered, such as poor recovery of monensin from the tissues, interference from fat and other co-extractives and, for liver, the presence of compounds producing zones of inhibition with the test organism. Some results are shown in Table 1, and these were considered to be satisfactory, although the limit of detection (0.0125 mg kg^{-1}) claimed by the authors could not be achieved with every sample. The layer of agar applied should be as thin as possible (50–80 ml per plate, 230 mm square). A plot of dose against response on a log - linear graph was a straight line in the range 0.2–0.05 μg of monensin. Below this level, the response was found to be erratic. Some participants reported that the zones observed were elliptical rather than circular.

Separation of Monensin from Other Ionophores

Narasin, salinomycin and lasalocid are very similar in chemical structure to monensin. The method of Okada *et al.*[5] failed to separate monensin from the other three compounds on the TLC plate. Two laboratories looked at modifications of the method involving (1) developing the TLC plate twice with ethyl acetate and (2) using water-saturated ethyl acetate as the development solvent.

The R_F values obtained are shown in Table 2. Both modified development systems clearly separate monensin from the other ionophores. Ethyl acetate saturated with water (system 2) gave a marginally better separation of monensin than the other ionophores, whereas ethyl acetate double development (system 1), although more time consuming, was able to resolve all four ionophores. Similar findings were published by Owles,[7] whose results are also included in Table 2 for comparison. However, the use of a fixed ratio of ethyl acetate to water (97 : 3 *V/V*) was thought to be preferable to the method recommended by Owles.[7]

Evaluation of the Method of Tihova and Peneva[6]

The authors claim that monensin reacts with *p*-anisaldehyde (4-methoxybenzaldehyde) in acidic solution to form a fluorescent compound that is more sensitive than that formed in the

Table 2. Separation of ionophores on a TLC plate

Developing solvent	Monensin	Salinomycin	Narasin	Lasalocid	Laboratory
	R_F values				
Ethyl acetate (twice)	0.25	0.43	0.48	0.50	2
Water-saturated ethyl acetate (once)	0.33	0.53	0.59	0.61	2
Ethyl acetate (twice)	0.30	0.51	0.58	0.76	3
Water-saturated ethyl acetate (once)	0.31	0.55	0.59	0.61	3
Water-saturated ethyl acetate (twice)	0.52	0.77	0.81	0.83	3
100 ml of ethyl acetate plus 3 ml of water[7]	0.38	0.51	0.60	0.71	Owles[7]
	R_x values				
Ethyl acetate (twice)	1.0	1.71	1.92	2.0	2
Water-saturated ethyl acetate (once)	1.0	1.61	1.79	1.85	2
Ethyl acetate (twice)	1.0	1.70	1.93	2.54	3
Water-saturated ethyl acetate (once)	1.0	1.77	1.90	1.97	3
Water-saturated ethyl acetate (twice)	1.0	1.48	1.56	1.59	3
100 ml of ethyl acetate plus 3 ml of water[7]	1.0	1.34	1.58	1.87	Owles[7]

Table 3. Recovery of monensin residues from fortified tissue. First collaborative trial

Laboratory	Tissue	Monensin added/mg kg^{-1}	Estimated recovery, %
1	Muscle	1.0	110–120
		0.6	90–95
		0.2	95–100
		0.1	95–100
		0.05	95–100
	Fat	1.0	50–60
		0.6	75–80
		0.2	125–130
		0.1	90–95
		0.05	90–95
	Liver	N.q.*	
2	Muscle	1.0	90–100
		0.6	90–100
		0.2	90–100
		0.1	90–100
		0.05	90–100
	Fat	1.0	90–100
		0.6	90–100
		0.2	90–100
		0.1	90–100
		0.05	90–100
	Liver	N.q.*	
3	Muscle	1.0	90–100
		0.6	90–100
		0.2	90–100
		0.1	90–100
		0.05	90–100
	Liver	1.0	65–85
		0.6	65–85
		0.2	65–75
		0.1	55
		0.05	55
	Fat	0.1	N.q.*
	Liver	0.1	70–80, 70–80 after clean-up
	Fat	0.1	70–80, 80–90 after clean-up
4	Muscle	0.2	100
		0.1	33–100
		0.05	100–200
	Muscle	1.0	N.q.
		0.6	N.q.
		0.2	N.q.
	Liver	0.2	N.q.
		0.1	N.q.
		0.05	N.q.
		1.0	N.q.
		0.6	N.q.
		0.2	N.q.

* Not quantifiable.

Table 4. Recovery of monensin residues from fortified tissue. Second collaborative trial

Laboratory	Tissue	Monensin added/mg kg^{-1}	Estimated recovery, %
1	Muscle	0.2	100
		0.1	100
		0.05	100
	Liver	0.2	60–65
		0.1	60–125
		0.05	0
	Fat	0.2	55–60
		0.1	35–40
		0.05	55–60
2	Muscle	0.2	80
		0.1	80
		0.05	80
	Liver	0.2	50
		0.1	50
		0.05	50
	Fat	0.2	25
		0.1	25
		0.05	25
3	Muscle		N.q.
	Liver	0.05–0.2	50
	Fat	0.2	50
		0.1	80
		0.05	50
4	Muscle	0.2	100
		0.1	67–100
		0.05	67
	Liver	0.2	67–100
		0.1	67–100
		0.05	67
	Fat	0.2	100
		0.1	100
		0.05	67

* Not quantifiable.

reaction with vanillin (4-hydroxy-3-methoxybenzaldehyde). However, they gave no directions for the formation of the colour apart from a general instruction to "warm the plate slightly for 2–3 min." Accordingly, the Sub-Committee initially examined the colour formation on the TLC plate.

Monensin reacts with the reagent to produce a complex that is visible in daylight and is also fluorescent under UV irradiation. At low temperatures the complex is yellow and the fluorescence is a maximum at 366 nm. At higher temperatures the complex is orange, although the wavelength of maximum fluorescence does not appear to change. Heating the plate above 80 °C for 5 min destroys the fluorescent compound, increases the background fluorescence of the plate and, above 100 °C, causes charring. Prolonged exposure to UV irradiation may cause the fluorescent spots to fade. Some collaborators obtained satisfactory results by heating the plate for 5 min at 60 °C. Other workers preferred to allow the colour to develop at room temperature over a 10–20 min period. The other ionophores also react with the reagent, but with lasalocid the reaction takes several hours at room temperature.

The method was then evaluated using a similar protocol to that designed for the bioautographic method. Preliminary work by three laboratories gave estimated recoveries in the range 80–100% and was sufficiently encouraging for a further study to be arranged using muscle, liver and fat tissues. The results obtained have been collected together in Table 3. In general, satisfactory recoveries were obtained from muscle tissue. Recoveries from liver tissue could not be determined unless an additional clean-up procedure was used. A second collaborative trial was therefore organised, in which participants were asked to use the column clean-up procedure in the method of Okada *et al.*[5] for fat and liver samples, in the hope that the spots on the TLC plate would be less distorted by the lipid material present in the extract applied to the plate. The results are shown in Table 4. It is clear that for levels of monensin of 0.1 mg kg^{-1} and below, the method is really only semi-quantitative. Finally, a third collaborative trial was then arranged in which participants were supplied with "blind" samples of chicken tissue, some of which had been fortified with monensin at a level of 0.1 mg kg^{-1}. The column chromatographic clean-up procedure was used for all three tissues and either the bioautographic or the TLC - spectrophotometric method at the end determination stage. The primary objective was to determine whether or not residues of monensin in chicken tissue could be reliably detected at the 0.1 mg kg^{-1} level. The results are shown in Table 5. Apart from two results from laboratory 2, correct identifications were made. Laboratory 2 subsequently repeated their work

Table 5. Detection of monensin residues in fortified chicken tissues. Third collaborative trial*

		Muscle				Liver		Fat	
		A	B	C	D	A	B	A	B
Fortified		N	Y	Y	N	Y	N	N	Y
Laboratory	Method								
2	Bioautograph	−	NS	+	−	+	−‡	?	NS
	Chemical	−	NS	+†	−	+	−	−	NS
3	Bioautograph	−	+	+	NS	?	−	NS	+
	Chemical	−	+	+	NS	+	−	NS	+
4	Chemical	NS	+	+	−	+	−	−	NS
6	Chemical	−	+	+	NS	+	−	NS	+

* ?, Ambiguous result; NS, sample not supplied; −, negative; +, positive; N, no; Y, yes.
† Negative in first test.
‡ Positive in first test.

and obtained satisfactory results as shown in the table. In general, recoveries were estimated at about 50%.

One member examined the use of HPTLC and found that the limit of detection was 10 ng as opposed to 100 ng by conventional TLC. However, the extraction and clean-up stages may not be able to cope at such low levels, and overall there was little advantage to be gained by using HPTLC.

Conclusions

Both methods examined were found to be semi-quantitative for the detection of monensin residues at levels below 0.1 mg kg^{-1}. The detection limits and recovery values claimed by the original authors could not be confirmed in this study. Neither method was thought to be significantly better than the other. Hence, both methods are given in the Appendix and are recommended by the Sub-Committee for the detection of monensin residues in chicken tissue. The analyst can make a choice based on the facilities available in his or her laboratory. Alternatively, as both methods use the same extraction and clean-up procedures, it may be advisable to use both end-detection systems to confirm positive findings in chicken tissues.

APPENDIX 1

Detection of Monensin Residues in Chicken Tissue. Bioautographic Method

Principle

Monensin is extracted with methanol and partitioned into carbon tetrachloride. After evaporation to dryness, the residue is purified on a silica gel column and examined by thin-layer chromatography using bioautography with agar innoculated with *Bacillus subtilis*.

Reagents

All reagents should be of analytical-reagent grade unless otherwise stated. **Caution.**—Attention is drawn to the toxic nature of some reagents. Suitable precautions should be taken.

Agar medium. Containing 10 g of glucose, 2.5 g of dried yeast extract, 0.69 g of K_2HPO_4, 0.45 g of KH_2PO_4 and 15 g of agar powder in 1000 ml. Sterilise and check that the pH is about 6. Further details of the preparation and use of agar can be found in Donoho and Kline[4] or in reference 8.

Carbon tetrachloride.

Chloroform. Remove any ethanol present as stabiliser by distillation, or by washing with water and drying over anhydrous sodium sulphate.

Developing solvent for TLC. Ethyl acetate - water, 97 + 3 (*V/V*).

Ethyl acetate.

Hexane.

Methanol.

Micro-organism. Bacillus subtilis ATCC 6633 (NCIB 8054).

Monensin. Available from Eli Lilly, Indianapolis, from the International Laboratory for Biological Standards, Central Veterinary Laboratory, Weybridge, Surrey, or from Sigma, Poole, Dorset.

Monensin standard solution, 10 ug ml^{-1} in methanol.

Silica gel. Kiesegel 60, Merck, or equivalent.

Sodium sulphate. Anhydrous.

Apparatus

Assay plates. 230 mm square (*e.g.*, Nunc) or equivalent, sterile and disposable.

Centrifuge. Capable of accepting 100-ml tubes.

Chromatography tank for TLC.

Glass columns. 250 × 11 mm fitted with a glass filter, stopcock and reservoir.

Homogeniser.

Rotary film evaporator.

Separating funnel, 250 ml.

Thin-layer plates. Kieselgel 60 without fluorescent indicator, pre-coated, 200 × 200 mm, 0.25 mm thick.

Procedure

Extraction

Liver and muscle. Add 30 g of tissue to a 100-ml glass centrifuge tube containing 60 ml of methanol. Homogenise and then centrifuge for 10 min. Extract the supernatant three times, each with 30 ml of carbon tetrachloride. Evaporate the carbon tetrachloride solution to dryness in a rotary film evaporator at 40 °C.

Fat. Add 30 g of sample to a 100-ml centrifuge tube containing 60 ml of methanol. Homogenise and then centrifuge for 10 min. Evaporate 40 ml of the supernatant to dryness *in vacuo* in a rotary film evaporator at 40 °C.

Clean-up

Prepare a suitable column by adding approximately 1.5 g of silica gel to a column containing hexane, to give a layer 12 mm deep. Stir to eliminate air bubbles and pack uniformly by tamping. Then add a 20-mm layer of anhydrous sodium sulphate on top of the column. Transfer the extracted residue to the prepared column using several 5 ml portions of hexane. Allow the liquid to fall to the level of the top of the packing. Wash the column with 40 ml of chloroform and elute monensin from the column using 25 ml of chloroform -

methanol (95 + 5, *V/V*). Evaporate the eluate to dryness in a rotary film evaporator and transfer the residue into a 10-ml test-tube using three portions of hexane (1–2 ml each). Remove the hexane by evaporation in a stream of air or nitrogen and take up the residue in 0.2 ml of methanol.

Thin-layer chromatography

Apply 20 µl of the prepared extract to a TLC plate 30 mm from the bottom edge. Apply, alongside the sample spot, equal volumes of a range of monensin standard solutions containing 0.05, 0.1 and 0.2 µg of monensin, respectively. Develop the chromatogram until the solvent front is 120 mm from the origin. Allow the solvent to evaporate from the plate in air. Melt the agar medium in boiling water, cool to about 60 °C and spray on to the surface of the plate. Prepare inoculated agar by cooling 100 ml of melted agar medium to about 60 °C and inoculate with 0.2 ml of *B. subtilis* spore suspension. Mix the inoculated agar gently and pour 50–80 ml evenly over the surface of the TLC plate contained in a suitable assay plate. Allow the plate to cool until the agar solidifies and then incubate at 37 °C for 16–18 h. Measure the longest and shortest diameters of each inhibition zone and, by reference to the standard spots, calculate the amount of monensin present in the extract.

APPENDIX 2

Detection of Monensin Residues in Chicken Tissue. Chemical Method

Principle

Monensin is extracted with methanol and partitioned into carbon tetrachloride. After evaporation to dryness, the residue is purified on a silica gel column and examined by thin-layer chromatography. A fluorescent derivative is prepared with *p*-anisaldehyde.

Reagents

All reagents to be of analytical-reagent grade unless otherwise stated. **Caution.**—Attention is drawn to the toxic nature of some reagents. Suitable precautions should be taken.

Acetic acid, glacial.

Carbon tetrachloride.

Chloroform. Remove any ethanol present as stabiliser by distillation, or by washing with water and drying over anhydrous sodium sulphate.

Developing solvent for TLC. Ethyl acetate - water, 97 + 3 (*V/V*).

Ethyl acetate.

Hexane.

Methanol.

Monensin. Available from Eli Lilly, Indianapolis, from the International Laboratory for Biological Standards, Central Veterinary Laboratory, Weybridge, Surrey, or from Sigma, Poole, Dorset.

Monensin standard solution. 10 µg ml^{-1} in methanol.

Silica gel. Kieselgel 60, Merck, or equivalent.

Sodium sulphate, anhydrous.

Sulphuric acid. Sp. gr. 1.84.

Detection reagent. Dissolve 0.5 ml of *p*-anisaldehyde (4-methoxybenzaldehyde) in 8.5 ml of methanol. Add 0.5 ml of sulphuric acid and five drops of acetic acid and stir.

Apparatus

Centrifuge. Capable of accepting 100-ml tubes.

Chromatography tank.

Glass columns. 250 × 11 mm i.d. fitted with a glass filter, stopcock and reservoir.

Homogeniser.

Rotary film evaporator.

Separating funnel, 250 ml.

Thin-layer plates. Kieselgel 60, without fluorescent indicator, pre-coated, 200 × 200 mm, 0.25 mm thick.

UV lamp. With a filter at 366 nm.

Procedure

Extraction

Liver and muscle. Add 30 g of tissue to a 100-ml glass centrifuge tube containing 60 ml of methanol. Homogenise and then centrifuge for 10 min. Extract the supernatant three times, each with 30 ml of carbon tetrachloride. Evaporate the carbon tetrachloride solution to dryness in a rotary film evaporator at 40 °C.

Fat. Add 30 g of sample to a 100-ml centrifuge tube containing 60 ml of methanol. Homogenise and then centrifuge for 10 min. Evaporate 40 ml of the supernatant to dryness *in vacuo* in a rotary film evaporator at 40 °C.

Clean-up

Prepare a suitable column by adding approximately 1.5 g of silica gel to a column containing hexane to give a layer 12 mm deep. Stir to eliminate air bubbles and pack uniformly by tamping, then add a 20 mm layer of anhydrous sodium sulphate on top of the column. Transfer the extracted residue into the prepared column using several 5-ml portions of hexane. Allow the liquid to fall to the level of the top of the packing, then wash the column with 40-ml of chloroform and elute monensin from the column using 25 ml of chloroform - methanol (95 + 5, *V/V*). Evaporate the eluate to dryness in a rotary film evaporator and transfer the residue into a 10-ml test-tube using three portions of hexane (1–2 ml each). Remove the hexane by evaporation in a stream of air or nitrogen and take up the residue in 0.2 ml of methanol.

Thin-layer chromatography

Apply 20 µl of the prepared extract to a TLC plate 30 mm from the bottom edge. Apply, alongside the sample spot, equal volumes of a range of monensin standard solutions containing 0.05, 0.1, 0.02 and 0.2 µg of monensin, respectively. Develop the chromatogram until the solvent front is 120 mm from the origin. Allow the plate to dry in air and spray with the detection reagent. Allow the plate to stand for 15 min at room temperature then examine it under UV light. Monensin appears as a yellow - orange spot with an R_F value of approximately 0.3–0.4. Calculate the amount of monensin present by reference to the standard spots.

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SHORT PAPERS

Simple Fibre Optic pH Sensor for Use in Liquid Titrations

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This paper describes the development of a fibre optic sensor incorporating an LED source and solid-state photodiode detection for the determination of the pH of solutions containing the indicator phenol red. The sensor can be used in, for example, liquid titration studies. This inexpensive and rapid response device is shown to have a reproducible characteristic output, with a low response change at elevated temperatures.

Keywords: *Optical pH sensor; optical fibre sensor; light-emitting diode source; phenol red*

The research field of fibre optic sensing is one that has seen significant growth in recent years and many fibre optic sensors for the measurement of important parameters in industrial situations have been described.[1] Hence, parameters such as temperature, pressure, fluid flow and liquid level are frequently measured by novel optical devices, but the sensing of chemical parameters is less frequently tackled by fibre optic means, and very few devices have been reported. This is surprising as some of the well known advantages of fibre optic transducers are particularly valuable for use in the environment of a chemical process. Fibre optic sensors are non-electrical, and as no currents are flowing at the sensor head there is no risk of explosion from sparks from short circuits. In most instances, there is no danger of causing ignition by the light used to address the sensor being coupled into the flammable material owing to its comparatively low level of emission. Most transducers could be constructed entirely from passive insulating materials, if necessary, and therefore they can be used in a corrosive atmosphere or solution in which some conventional sensors would be attacked or would be bulky due to the addition of protective coatings or casings. Additionally, if the chemical process to be studied involves the use of high magnetic or electrical fields, the optical sensor is unaffected by their presence and the fidelity of the transmitted signal is preserved.

In this paper, a simple, inexpensive fibre optic pH sensor is described for use in the sensing of liquid solutions to which an indicator dye has been added, for example, in acid - base titrations. The indicator dye changes colour as a result of a change in the optical absorption spectrum. This sensor determines the change quantitatively and hence the pH of the solution over a pre-determined range. Previous work in this area has concentrated on, for example, a narrow pH sensor for biological applications,[2] a flow cell device with a wider pH measurement function[3] and an immobilised indicator on a polymer base connected to a plastic fibre bundle. These devices use bulky, conventional lamp sources and optical filters. Peterson *et al.*[2] used a mechanical filter cycling wheel to determine a reference signal. The devices of Kirkbright *et al.*[3,4] required extensive and expensive optical processing, using a grating monochromator and photomultiplier to detect the returned signal. A fluorescence monitoring pH sensor[5] again used large, expensive optical components, such as a xenon arc lamp, monochromator and a commercial spectrofluorimeter. The use of such hardware severely increased costs, thereby reducing the advantage of cheap fibre optical components.

The device described here is designed with simplicity of components included as a key feature. A small, bright light-emitting diode is used as the source and detection is with a Si p - i - n diode. The use of these components means that this sensor is comparatively inexpensive, with a very rapid response, and can be packaged in such a way as to be portable and, if necessary, battery operated. Such a pH meter, with an interchangeable optical probe, could then be a direct substitute for the small, pocket-sized electrical pH meters available for use in titration studies.

Experimental

The device is illustrated schematically in Fig. 1. An ultra-bright green LED (RS components) with a peak wavelength of 565 nm (40 nm full width at half-maximum) and output intensity of 120 mcd is modifided by polishing the clear plastic lens flat to within *ca.* 1 mm of the emitting semiconductor chip. This is then mounted in a standard SMA fibre optic device housing (T018 size, Radiall). The light emitted is launched into a fibre bundle (B_1) consisting of two 600 μm diameter core fibres (PCS 600, Quartz et Silice) and one 200 μm diameter core fibre (PCS 200) held together in a modified SMA connector barrel. This 2 m long bundle is separated after the connector, with the narrow fibre (F) being led to a Si p - i - n diode (D_1) (BPX 65) and the two 600 μm fibres being led to the input end of the sensor. The large size of the LED emitting surface (*ca.* 1.5 mm across) enables light to be coupled into each fibre. The sensor head is manufactured in this instance from a small cylinder of stainless steel with an internal length of *ca.* 1 cm. The end of the cylinder is closed with a threaded stainless-steel cap, which is polished to provide a high level of reflectivity. Both ends are demountable for cleaning and

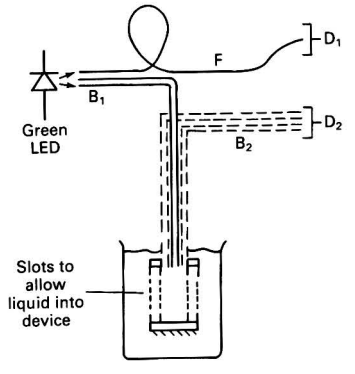

Fig. 1. Schematic diagram of the sensor

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re-polishing, if necessary. As polished stainless steel is used, this can readily be cleaned after use and re-polishing should therefore be infrequent. Should this happen, re-calibration can easily be performed with a solution of pH 7 or 9, with reference to the calibration graph. At the input end of the cylinder, four further 600 μm diameter fibres, arranged around the two input fibres, collect light that has passed through the solution twice, having been reflected by the polished end-cap. This fibre bundle (B_2) is led to the input of a second Si p - i - n diode (D_2), where the light intensity level is determined. The device is used by placing the sensor head in the solution containing the indicator dye and the absorption characteristic of the indicator, averaged over the narrow emission band of the LED, is determined.

The fibre bundles used were made from individual fibres, stripped of their plastic coating at the end and cemented with Epotek 353ND epoxy resin. The SMA connector barrels and the six fibre inputs of the sensor head were constructed in this way and a polished surface was obtained at the fibre interfaces by conventional means, in order to ensure good optical coupling characteristics. The resin - silica fibre combination is inert and is resistant to both strongly acidic and alkaline solutions. The sensor cylinder itself could be made from a plastic material, *e.g.*, PTFE or nylon, when solutions in which the stainless steel is attacked are investigated.

The electrical design of the device is described below. A simple configuration is employed, in which the light from the 200 μm diameter fibre is detected and used to monitor any change in the emissivity of the LED. Such ultra-bright diodes must be operated close to the maximum current (*ca.* 30 mA) and therefore a gradual change in the emission level may occur, so it is important to monitor this directly with a fibre optic connected to the main input fibre bundle (B_1). In normal use lengths of able above a few metres are not necessary for a laboratory instrument, and as a fibre bundle is used it is preferable to design the sensor with the required length of cable. However, this does not preclude an initial design with long bundles for use in remote sites. The output from each detector is amplified with conventional integrated circuit devices and the signal levels are displayed in this instance on digital voltmeters. The ratio of these signals reflects the pH of the solution.

Indicator and Reagents

The indicator dye used in this work was phenol red (phenolsulphonphthalein). This was chosen because it can be used over a comparatively wide range of pH and has a strong absorption feature at the peak emission wavelength of the LED. It exists in two tautomeric forms, which results in the sizes of its two absorption peaks varying in response to the pH of the solution. The absorption spectrum in the range 350–600 nm is shown in Fig. 2 for four sample solutions of pH 6.8–9.7, using a 1-cm absorption cell in a conventional spectrophotometer. This shows clearly the peak that is monitored in this device, at *ca.* 565 nm. The device was calibrated against a conventional electronic pH meter (Kent EIL 3055) referenced to standard buffer solutions, which were carefully made in the usual way. Solutions and chemicals were purchased from BDH Chemicals.

Solutions were prepared containing *ca.* 0.5 ml of indicator and the calibration was performed in a solution, stirred continuously, which contained the fibre optic probe and the conventional electronic meter probe. Measurements were performed in ambient light, although the beaker containing the solution was wrapped in an opaque layer. The construction of the probe is such that the fibre bundle at the sensor head is shielded from direct light. For use in very bright environments, the LED may be modulated electronically and discrimination against the unmodulated background light can be performed easily in the signal processing circuitry.

Calibration

The response of the device is shown in Fig. 3, with the ratio of the signals measured by the two photodiodes plotted as a function of the pH of the solution. Excellent reproducibility is shown by the coincidence of three successive experimental runs. The non-linear response is not a problem as this calibration may conveniently be stored in, and re-called from, a simple microprocessor. The resolution of the sensor is ±0.05 pH unit, arising from the inaccuracy in the measurement of the absorption peak, in the approximately linear region of the device response. The stability of the reading is good, <0.5% variation over a period of 1 h for a constant solution.

A major advantage of this sensor is that its response time to changes in the pH of the solution is very rapid (and diffusion limited only) owing to the optical method of measurement with detectors with a sub-microsecond response. This contrasts with the conventional electronic meter, which requires several seconds to readjust and show the same change. The temperature coefficient may be expressed as a change of pH per degree and was determined as 0.013 pH unit K^{-1} over the temperature range 24–40 °C around pH 7.5. This compares closely with the results from other workers (0.017, phenol red[2]; 0.013, bromothymol blue[3]) for indicator dyes and is significantly better than that seen with a conventional glass electrode.

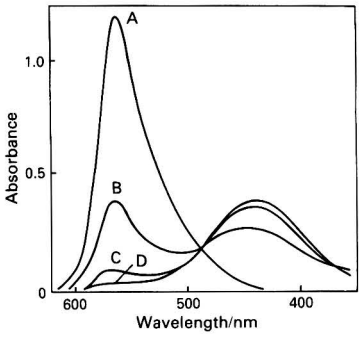

Fig. 2. Absorption spectrum of the indicator in solutions of different pH. A, 9.7; B, 8.0; C, 7.2; and D, 6.8

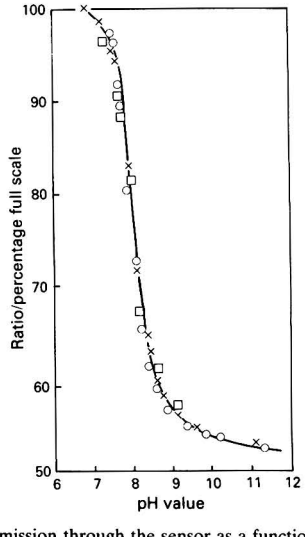

Fig. 3. Transmission through the sensor as a function of the pH of the indicator solution for three separate experimental runs. □, First run; ○, second run; and ×, third run

Conclusion and Future Work

This study has demonstrated a simple, inexpensive fibre optic pH sensor for use in liquid solutions. The opto - electronic components used were an LED and Si p - i - n detectors, with simple signal processing and no optical filtering elements. A rapid response sensor results, which offers considerable benefits in terms of cost, safety, reliability and applicability to certain corrosive environments. Additionally, a source monitoring reference channel, incorporated in the input fibre bundle, is included to correct for possible source fluctuations.

Future developments lie in two directions. Work is proceeding towards the inclusion of a reference channel, the fibre optic of which is fully incorporated in the input and output optical connectors of the sensor itself. Alternatively, a balanced intensity approach to referencing using two separate fibre paths for the modulated and reference signal, with both signals transmitted through both channels, can be used.[6] A second optical source at the same wavelength as the first is used and the output does not (theoretically) depend on source intensities, fibre and connector attenuations or detector sensitivities. Further, microprocessor techniques may be used to calculate the signal ratios involved directly. Even with the inclusion of such additional features, the cost of the sensor can still be kept comparatively low and yet a sophisticated and accurate device produced. Such a sensor has a temperature characteristic superior to that of a conventional electronic meter and, in contrast to some proposed fibre optic pH sensors, will not be affected by parameters such as humidity changes in the air or a small change in ambient temperature.[7]

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Titrations in Non-aqueous Media

Part II.* Basicity Order of Aliphatic Amines in Nitrobenzene Solvent

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The relative basicity order of methyl-, ethyl-, propyl- and butylamines has been determined potentiometrically with perchloric acid in nitrobenzene solvent and found to be $R_3N > R_2NH > RNH_2 > NH_3$, where R = Et, n-Pr or n-Bu. However, for the methylamines, the order is $Me_2NH \geq Me_3N > MeNH_2 > NH_3$. The orders in primary, secondary and tertiary amines are $EtNH_2 > MeNH_2 > n\text{-}PrNH_2 > n\text{-}BuNH_2 > NH_3$; $Et_2NH > Me_2NH > n\text{-}Pr_2NH > n\text{-}Bu_2NH > NH_3$; and $Et_3N > n\text{-}Pr_3N \geq n\text{-}Bu_3N > Me_3N > NH_3$. These results show that, in general, an increase in the number of alkyl groups increases the basicity of the amine, and that an increase in the size of the alkyl group decreases the basicity. n-Butylamine is a stronger base than branched-chain primary butylamines.

Keywords: *Non-aqueous titration; potentiometric titration; amines; nitrobenzene solvent; basicity order*

The basicity orders of amines have been a source of considerable confusion for many years,[1-12] as there are many factors which influence basicity. The factors influencing the basicities of aliphatic amines are relatively limited, although the most significant factors, such as molecular properties and solvent effects, are still operative.

In the work reported in this paper, the basicities of aliphatic amines have been determined in nitrobenzene solvent by potentiometric titration with perchloric acid, one of the strongest non-aqueous media acids available. Four different series of aliphatic amines have been titrated with perchloric acid, namely methyl-, ethyl-, n-propyl- and n-butylamine. In addition to these, isopropylamines and some branched-chain butylamines have also been titrated. All the amines showed good S-shaped potential *vs.* mequiv. acid or mequiv. base (milliequivalent of acid or milliequivalent of base) titration graphs. An example of the titration graphs for n-butylamines is given in Fig. 1.

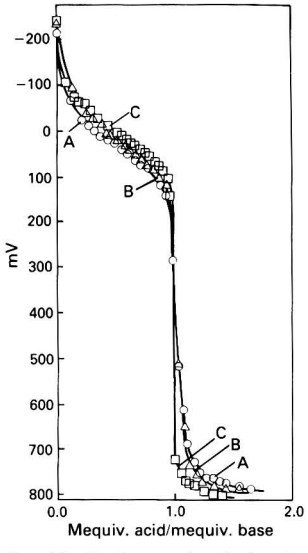

Fig. 1. Potentiometric titration graphs of n-butylamines with perchloric acid in nitrobenzene. A, $n\text{-}BuNH_2$; B, $n\text{-}Bu_2NH$; and C, $n\text{-}Bu_3NH$

As is evident from the shapes of the titration graphs, no homo-conjugation reactions took place during the titrations.[1,2] This indicates that nitrobenzene is a good solvent for ionic organic compounds. Half-neutralisation potentials have been determined from the titration curves.

As the half-neutralisation potentials of the amines are slightly concentration dependent (we have carried out a number of experiments in this area), the titrations were carried out with dilute solutions (0.001 M). Also, in order to minimise the errors that would originate from the dilution of solutions during titrations, 0.034 M perchloric acid was used. The end-points were reached after the addition of about 0.5 ml of acid solution. This amount of acid solution allowed at least fifteen readings to be taken before the end-point of the titration was reached.

Half-neutralisation potentials of the amines *versus* the number of alkyl groups on the amines have been plotted in Fig. 2. It can be seen that there is a fairly good correlation between the basicity of amines and the number of alkyl groups in the amine series, with the exception of the methyl series. Ethylamines are the most basic compounds of the amines.

Isopropylamine and branched-chain butylamines are not included in Fig. 2.

Another set of experiments was performed in a nitrobenzene - light petroleum (1 + 3) system. All the experiments were repeated under identical conditions and almost the same series order was found. The anomaly observed in the methylamine series remained unchanged.

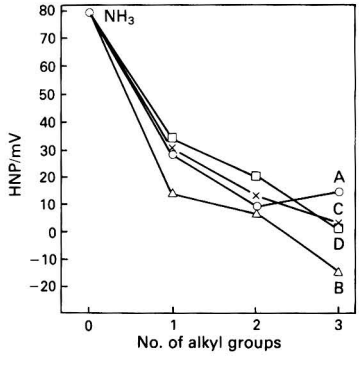

Fig. 2. Half-neutralisation potentials of the aliphatic amines in nitrobenzene *versus* number of alkyl groups. A, Methyl; B, ethyl; C, n-propyl; and D, n-butyl

* For Part I of this series, see *Analyst*, 1986, **111**, 949.
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Experimental

Apparatus and Chemicals

Potentiometer and accessory. An Orion Model 801 A digital pH meter equipped with glass and calomel electrodes was used throughout this work. The saturated KCl solution of the calomel electrode was removed and the electrode washed several times with anhydrous methanol. After drying, the electrode was then filled with saturated KCl solution in non-aqueous methanol. A pressure of 20 mmHg was applied to the solution in the calomel electrode in order to prevent the diffusion of the solution into the electrode. After each titration the electrode was washed twice with anhydrous methanol to remove nitrobenzene solution from the surface of the electrodes. Pure anhydrous methanol dries easily without leaving any stains. Before using again, electrodes were dipped into pure nitrobenzene solvent to remove any traces of methanol.

A magnetic stirrer was used in the titrations and titrations were carried out in a 50-ml beaker wound with copper wire. The copper wire and all other electrical equipment were earthed.

A semi-microburette, which could be read to 0.01 ml, was used for the titrations.

Nitrobenzene. Nitrobenzene (Merck) was used after purification in the following way: 10 g of P_2O_5 were introduced into 1 l of nitrobenzene. After shaking, the mixture was left overnight and then distilled twice at reduced pressure by the aid of a suction pump equipped with a liquid air-cooled trapper. The solvent prepared in this way was straw-yellow and its refractive index was 1.5513.

Purified nitrobenzene is a fairly good solvent for molecular and some ionic compounds. It has a large titration interval extending from −700 mV (basic side) up to +800 mV (acidic side).

Perchloric acid solution. Anhydrous 0.034 M perchloric acid solution was used in all the titrations. This was prepared by taking 0.072 ml of 70% perchloric acid (Merck) with a micro-pipette and adding this dropwise to 5 ml of ice-cooled pure acetic anhydride. At higher temperatures, a vigorous reaction takes place and a dark brown solution is obtained instead of a light yellow solution. The resulting light yellow solution was left for 5–6 h at room temperature, and then 1.00 ml of solution was taken from it and introduced into a 50.0-ml calibrated flask before being diluted to 50.0 ml by the addition of nitrobenzene. The concentration of the final solution was determined against a primary standard, diphenylguanidine, and was found to be 0.034 M. This solution was stable for 3–4 months, if kept under refrigeration in a dark flask.

Amines. The amines used were all of analytical-reagent grade. Diethylamine was obtained from Riedel de Häen and the others from BDH Chemicals. Methylamines were prepared from their hydrochlorides. Each hydrochloride was treated with aqueous sodium hydroxide and the gas evolved was passed over dry NaOH and then introduced directly to the solvent.

Amine solutions were titrated immediately after preparation.

Results and Discussion

Until now, no simple explanation of the basicity orders of aliphatic amines has been given.[2,4–17] This is probably because of the number of factors that influence the basicity of the amines.

Potentiometric titrations of ammonia solution and aliphatic amines with perchloric acid in nitrobenzene have shown some interesting and unexpected relative basicity orders: $Et_3N > Et_2NH > EtNH_2 > NH_3$; $n\text{-}Pr_3N > n\text{-}Pr_2NH > n\text{-}PrNH_2 > NH_3$; $n\text{-}Bu_3N > n\text{-}Bu_2NH > n\text{-}BuNH_2 > NH_3$. These relative basicity orders are identical with the orders found by other workers in the gas phase.[5,11,12,15,18] (It is very difficult to give a theoretical explanation of these similarities, because one set of experiments were carried out in the gas phase and the other set of experiments were carried out in the condensed phase. In the gas phases intrinsic electronic factors affect the basicity without the interference of the solvent.)

There are also interesting orderings among the primary, secondary and tertiary amines, although these orderings are in conflict with the results of other workers[5,7–12]: $EtNH_2 > n\text{-}PrNH_2 > n\text{-}BuNH_2 > NH_3$; $Et_2NH > n\text{-}Pr_2NH > n\text{-}Bu_2NH > NH_3$; $Et_3N > n\text{-}Pr_3N \geqslant n\text{-}Bu_3N > NH_3$.

For the methylamine series, a different order was found: $Me_2NH > Me_3N > MeNH_2 > NH_3$. This order unexpectedly parallels the order found in water by Brown.[4] In water there are two main opposing effects influencing the basicities of the amines, namely hydration and inductive effects. The hydration effect decreases with an increase in the number of alkyl substitutions, whereas the inductive effect increases with an increase in the number of alkyl substitutions. However, in nitrobenzene solvent, this explanation is not applicable.

The order determined for the methylamines disagrees with the findings of Benoit and co-workers in dimethyl sulphoxide.[9,10]

The relative basicity orders of primary, secondary and tertiary amines, including methylamines, are as follows: $EtNH_2 > MeNH_2 > n\text{-}PrNH_2 > n\text{-}BuNH_2 > NH_3$; $Et_2NH > Me_2NH > n\text{-}Pr_2NH > n\text{-}Bu_2NH > NH_3$; $Et_3N > n\text{-}Pr_3N > n\text{-}Bu_3N > Me_3N > NH_3$.

The extended primary and secondary amines, including isopropylamine and branched-chain butylamines, give the following orders: $EtNH_2 > MeNH_2 > iso\text{-}PrNH_2 > n\text{-}PrNH_2 > n\text{-}BuNH_2 > sec\text{-}BuNH_2 > tert\text{-}BuNH_2 > iso\text{-}BuNH_2 > NH_3$; $Et_2NH > Me_2NH > n\text{-}Pr_2NH > n\text{-}Bu_2NH > iso\text{-}Pr_2NH > NH_3$. Unfortunately, a good theoretical explanation as to why ethylamines are stronger bases than their methyl analogues has not been found.

Conclusions

From the investigations described above, the following conclusions can be drawn.

(1) *F*-strain seems to be very likely because any ethylamine is a stronger base than its propyl and butyl analogues.[4,16,19] $EtNH_2 > PrNH_2$; $Et_2NH > Pr_2NH$; $Et_3N > Pr_3N$. Identical orderings hold true for butylamines.

(2) *B*-strain is not observed in this work because tri-n-propylamine is more basic than n-propylamine. The same sequence is true also for tri-n-butylamine and n-butylamine.

(3) Basicity decreases with increase in size of the alkyl group, with the exception of methylamines. This finding is the opposite of findings reported in the literature.[7,9–12]

(4) n-Butylamine is more basic than the branched-chain butylamines. This is also in disagreement with data in the literature.[7,10–12]

(5) It is concluded that the order of basicities of amines may be entirely opposite when the titrations are carried out in different media.[7–12,19]

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Titrations in Non-aqueous Media

Part III.* Basicity Order of Aniline, *N*-Alkyl- and *N*-Aryl-substituted Anilines and Pyridine in Nitrobenzene Solvent

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The relative basicity order of ammonia, pyridine, aniline and *N*-methyl-, *N,N*-dimethyl-, *N*-ethyl-, *N,N*-diethyl-, *N*-aryl- and *N,N*-diaryl-substituted anilines have been determined potentiometrically with perchloric acid in nitrobenzene solvent and found to be $NH_3 > Py > PhNEt_2 > PhNMe_2 > PhNHEt > PhNHMe > PhNH_2 > Ph_2NH > Ph_3N$. This order in general conflicts with the results observed by other workers in either the gas phase or in the condensed phase. *N*-Alkyl substitution increases the basicity of the aniline, and *N*-aryl substitution decreases its basicity. Moreover, the number and size of the substituent influence the basicity of aniline. *N*-Ethyl-substituted anilines are more basic than the corresponding *N*-methyl-substituted anilines. The position of pyridine in the order is surprising and difficult to interpret.

Keywords: *Non-aqueous titration; potentiometric titration; anilines; nitrobenzene solvent; basicity order*

It is well known that two main effects influence the acidity or basicity of molecules, namely, structural and solvent (medium) effects.[1,2] Unfortunately, in most molecules, there are two or more structural effects and it is usually difficult to decide how much each contributes to the acidity or basicity of the molecules. Small differences in acidity or basicity between similar molecules are also extremely difficult to interpret, and one must be very careful in deciding which structural effect is the main influence on the acidity or basicity.

Solvent or medium effects are also very important, and the acidity or basicity of a molecule usually varies when its solvent is changed. For example, the basicity orders of n-butylamines against the Brönsted acids are $Bu_3N > Bu_2NH > BuNH_2$ in chlorobenzene solvent,[3] $Bu_2NH > Bu_3N > BuNH_2$ in benzene solvent[3] and $BuNH_2 > Bu_2NH > Bu_3N$ in cyclohexane solvent.[4] Hence a change of solvent may completely reverse the basicity order.

The relative basicity orders of *N*-alkylated and *N*-arylated anilines, including pyridine, have been determined in different solvents or media,[5–7] but have not as yet been determined in nitrobenzene solvent. In this work, the relative basicity orders of ammonia and eight amines have been determined potentiometrically with perchloric acid in nitrobenzene solvent. The amines titrated were pyridine (Py), aniline ($PhNH_2$), *N*-methylaniline (PhNHMe), *N,N*-dimethylaniline ($PhNMe_2$), *N*-ethylaniline (PhNHEt), *N,N*-diethylaniline ($PhNEt_2$), diphenylamine (Ph_2NH) and triphenylamine (Ph_3N). Titration graphs of these are given in Fig. 1.

As is evident from the titration graphs, no homoconjugation reaction took place during the titrations and all but diphenyl- and triphenylamine gave good S-shaped potential *vs.* mequiv. acid or mequiv. base graphs. Half-neutralisation potentials of the amines have been determined by means of the graphs. Half-neutralisation potentials of the diphenyl- and triphenylamines, which did not give proper titration graphs, have been determined stoicheiometrically, *i.e.*, the volumes of the titrant acid needed to reach the equivalence points have been calculated from the milliequivalent numbers of the titrated samples, and then the potentials at the half-volumes of the acid used have been taken as half-neutralisation potentials.

As the half-neutralisation potentials are slightly concentration dependent, titrations have been carried out on solutions with identical concentration, and also on dilute solutions (0.001 M).[8,9] In order to minimise the errors that might arise from the dilution of the solutions during the titrations, 0.034 M perchloric acid was used.

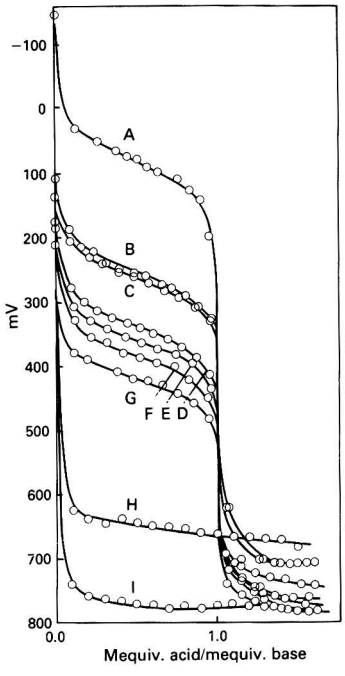

Fig. 1. Potentials *versus* mequiv. acids/mequiv. base. Base: A, ammonia; B, pyridine; C, *N,N*-diethylaniline; D, *N,N*-dimethylaniline; E, *N*-ethylaniline; F, *N*-methylaniline; G, aniline; H, diphenylamine; and I, triphenylamine

Experimental

Apparatus

All apparatus used was as under *Potentiometer and accessory* in Part II, and the electrode was washed and filled in an identical manner.

Chemicals

Nitrobenzene and perchloric acid were prepared and stored as described in Part II.

Ammonia. Ammonia was generated from NH_4Cl (Merck, Pure).

* For Part II of this series, see p. 1099.
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Pyridine. Pyridine was purchased from Merck (Pure) and was used without further purification.

Aniline and N-*methylaniline.* Aniline and *N*-methylaniline were purchased from Riedel de Häen (Pure) and were used without further purification.

N,N-*Diethylaniline. N,N*-Diethylaniline was obtained from Riedel de Häen (für Analyse) and was distilled twice under vacuum before use.

N,N-*Dimethylaniline. N,N*-Dimethylaniline was purchased from Fluka (Puriss) and was used without further purification.

Diphenylamine. Diphenylamine was purchased from BDH Chemicals (Indicator) and used without further purification.

Triphenylamine. Triphenylamine was purchased from BDH Chemicals (GPR) and used without further purification.

All amines and ammonia solutions were titrated immediately after preparation.

Results and Discussion

It is generally held that the relative basicity order of amines in a series does not change with a change of Brönsted acid titrant in the same solvent because the proton itself is too small to cause any appreciable steric hindrance.[10] Therefore, the differences observed in the relative basicity order of the same series, titrated with different Brönsted acids in different solvents, can be attributed to the change of the solvent. For this reason, when comparing the relative order of the amines in a series, only the change of solvent is taken into account.

As can be seen from the titration graphs obtained in nitrobenzene solvent given in Fig. 1, ammonia is more basic than the eight amines titrated in this work, and is followed by pyridine. Including ammonia, the over-all order of the amines is $NH_3 > Py > PhNEt_2 > PhNMe_2 > PhNHEt > PhNHMe > PhNH_2 > Ph_2NH > Ph_3N$ (I). All *N*-alkyl-substituted anilines are more basic and all *N*-aryl-substituted anilines are less basic than aniline.

More useful relative basicities can be extracted from sequence (I) above: $PhNMe_2 > PhNHMe > PhNH_2$ (II); $PhNEt_2 > PhNHEt > PhNH_2$ (III); $PhNH_2 > Ph_2NH > Ph_3N$ (IV); $Py > PhNMe_2 > PhNHMe$ (V); and $NH_3 > Py > PhNH_2$ (VI).

As is evident from Fig. 1, diphenylamine and triphenylamine do not give clear end-points.

The order (I) shows that *N,N*-diethylaniline is a stronger base than *N*-ethylaniline and *N,N*-dimethylaniline is a stronger base than *N*-methylaniline in nitrobenzene solvent. Moreover, *N*-ethyl-substituted anilines are more basic than their *N*-methyl-substituted aniline analogues. This means that an increase in the number or in the size of an alkyl group increases the basicity of the aniline. This is in good agreement with the results found by Brauman and co-workers in the gas phase with other compounds.[5,6,11]

The order (II) disagrees with the order $PhNH_2 > PhNHMe > PhNMe_2$ found by Benoit *et al.* in dimethyl sulphoxide solvent.[7] In addition, the order (V) also disagrees with the order $PhNH_2 > Py > PhNHMe > PhNMe_2$ found by the same workers.[7] Moreover, sequence (V) is also at variance with the order $PhNMe_2 > Py > PhNHMe > PhNH_2$ observed by other workers in the gas phase,[12,13] and the order (IV) does not fit the order $Ph_3N > PhNH_2$ obtained by others in the gas phase.[5,14] According to Dzidic,[5] the phenyl group is electron-donating relative to the proton in the gas phase, whereas it is electron-attracting relative to a saturated system. On the other hand, the order (V) is consistent with the order $Py > PhNH_2$ observed in the gas phase by other investigators.[5,12,13]

As is evident from order (I), pyridine is more basic than even *N,N*-dialkyl-substituted anilines, which are expected to be highly basic.[15–17] Hence, pyridine acts in nitrobenzene as if it was in the gas phase. This is a surprising observation, because the general belief is that the sp^3 hybrid orbital is more basic than the sp^2 hybrid orbital. As the nitrogen atom in pyridine is sp^2 hybridised, it should show a lower basicity than aniline, which is sp^3 hybridised.

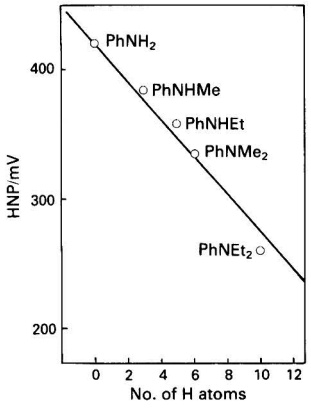

Fig. 2. Half-neutralisation potentials of amines *versus* number of hydrogen atoms

The higher basicity of pyridine relative to aniline in the gas phase can be interpreted as intrinsic basicity (in the absence of solvent effects), but the same property observed in nitrobenzene is very difficult to interpret. This is because nitrobenzene is not an inert solvent, and yet it has no similarity with the gas phase. It has a dielectric constant of 36.

Hence, the gas-phase basicities of the amines may not be diagnostically useful in studying the basicities of the compounds.

The order (VI) is the reverse of the result $Py > NH_3$ found by Dzidic in the gas phase.[5]

The order between pyridine and aniline in dimethyl sulphoxide is in the opposite sense ($PhNH_2 > Py$) to that in nitrobenzene solvent. This is another unexpected result, as dimethyl sulphoxide and nitrobenzene solvents are not at first sight so different in character as to reverse the order of basicity.

Finally, another surprising result is the regular increase in basicity of the anilines with the number and the size of *N*-alkyl substitutions, *e.g.*, $PhNEt_2 > PhNMe_2 > PhNHEt > PhNMe > PhNH_2$. In this series, if the number of hydrogens in aniline is arbitrarily taken to be zero, then the number of hydrogens in *N*-methyl-, *N*-ethyl-, *N,N*-dimethyl- and *N,N*-diethylanilines becomes 3, 5, 6 and 10, respectively. When the number of hydrogen atoms is plotted *versus* the half-neutralisation potentials of the amines, a nearly straight line is obtained (Fig. 2).

This relationship is very difficult to explain. It is as if each carbon–hydrogen bond gives a small part of its electron density to the nitrogen atoms of the amine. An increase in basicity implies a greater polarisability of the lone pair of electrons on the nitrogen atom, and a greater polarisability means that the lone pair of electrons on nitrogen is more easily donated. This results in a stronger attraction of the proton, and hence a greater basicity.

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Problems in the Dissolution of Silicates by Acid Mixtures

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The decomposition of silicate rocks using mixtures of mineral acids has been studied. The conventional acid method of dissolving rocks with hydrofluoric - perchloric - nitric acid mixtures is unsatisfactory when pyrophyllite is present. This mineral is not destroyed by acid attack, whereas other silicates such as mica, kaolinite, quartz, feldspars, smectite and interstratified illite - smectite are decomposed. If the acid attack is carried out in a pressure vessel, pyrophyllite is dissolved completely, but with more difficulty than the other silicate minerals present.

Keywords: *Pyrophyllite; silicate dissolution; acid mixtures*

One of the main concerns of silicate analysis over the years has been the development of a procedure to decompose the silicate minerals. Conventional methods used are based on the use of acids or fusion techniques. Various workers[1–4] have described schemes for the total decomposition of silicates using a hydrofluoric - perchloric - nitric acid mixture. Clays are composed mainly of silicate minerals and are usually easily decomposed by this mixture of acids. However, we have found that when pyrophyllite is present in a clay, dissolution is incomplete and a white residue is left after reaction. Similar problems occur when the samples are attacked with a hydrofluoric - sulphuric - nitric acid mixture.

In this work we studied the decomposition of clay samples by acid mixtures when pyrophyllite is present in varying proportions.

Experimental

Methods of Acid Attack

Method 1

Samples were treated as described by Bennett *et al.*[5] Weigh 0.250 g of the finely ground samples, add 5 ml of HNO_3 (1 + 4), 5 ml of $HClO_4$ (1 + 4) and 10 ml of HF (40% *m/m*) and evaporate to dryness. Cool the residue, add 5 ml of $HClO_4$ (1 + 4) and evaporate to dryness again. To the cool, dry residue, add 1–2 ml of HCl (37% *m/m*) and distilled water (approximately 25 ml) and warm. Cool, centrifuge, filter and wash several times with distilled water. Determine the different elements in solution by atomic absorption spectrometry.

Method 2

Transfer 0.20 g of finely ground samples into a PTFE-lined bomb, add 0.25 ml of HNO_3 (65%), 0.75 ml of HCl (37%) and 5 ml of HF (40% *m/m*) and heat at 50, 100, 125 and 140 °C for 60 min each. Cool to room temperature, dilute with distilled water, add 5 g of H_3BO_3 and heat at 60 °C in a plastic beaker until the sample has dissolved. Dilute the sample to 250 ml in a plastic flask with distilled water.

Silicate Analysis

Phosphorus was determined by the spectrophotometric method of Murphy and Riley[6] and the other elements present were determined by atomic absorption spectrometry.

Mineralogy

The mineralogical composition of the samples was determined using a Siemens X-ray diffractometer with Cu Kα radiation.

Results and Discussion

The major minerals present in the samples as deduced by X-ray diffraction are pyrophyllite, mica, kaolinite, feldspars and rutile; some quartz, smectite and interstratified mica - smectite are also present.

The samples, after treatment with HF - HNO_3 - $HClO_4$ acid mixtures, were not completely dissolved as is usual in clay minerals, and a white residue was left that did not dissolve even after several treatments. It is evident from these results that the triacid treatment does not completely dissolve these particular samples. The concentrations of the elements liberated by this method are shown in Table 1.

Table 1. Results of chemical analysis. A, Raw materials attacked by digestion bomb; B, raw materials attacked by HNO_3 - $HClO_4$ - HF mixture; and C, residues of B dissolved in the digestion bomb. Results quoted as percentage of the total mass of the sample

Element (quoted as oxide)	Sample 1			Sample 2		
	A	B	C	A	B	C
SiO_2	54.79	—	65.50	90.22	—	—
Al_2O_3	33.13	21.26	28.09	4.73	3.36	67.68
Fe_2O_3	0.23	0.23	—	0.86	0.80	29.05
TiO_2	1.25	0.10	6.02	0.69	0.05	3.15
CaO	0.26	0.25	—	0.93	0.93	—
MgO	0.25	0.25	—	0.07	0.07	—
Na_2O	0.78	0.76	—	0.10	0.10	—
K_2O	2.76	2.75	—	0.27	0.27	—
P_2O_5	0.15	0.14	—	0.02	0.02	—
Loss on ignition	5.93			1.95		
Total	99.53			99.84		

After the original samples and the residues from the acid method had been attacked in a digestion bomb at 140 °C for 60 min, the dissolutions were complete in all instances. The total element concentrations and the proportions of elements dissolved by the acid mixtures are shown in Table 1.

The mineralogical composition of the two samples in Table 1 are different. Sample 1 is composed of pyrophyllite (40%), mica (52%), kaolinite (5%), feldspar (<5%) and trace amounts of interstratified illite - smectite, whereas sample 2 is composed of pyrophyllite (5%), mica (20%) and quartz (75%). However, although the mineralogical composition of the two samples is different, the elements remaining undissolved after the triacid attacks are the same, although in different proportions. Iron, calcium, magnesium, potassium and phosphorus are completely dissolved by the acid treatment, whereas silicon, aluminium and titanium are dissolved only in part.

The proportion of SiO_2 and Al_2O_3 remaining undissolved in both samples is different, but the ratio between the two oxides in the residues (Table 1) is very similar (*ca.* 2.33). These results suggest that the residues have a similar composition. The X-ray diffraction results show that the material not dissolved is composed of pyrophyllite accompanied by rutile.

In order to determine the influence of the different acids used in the attacks, another sample (sample 3) composed of mica (68%), kaolinite (13%), feldspar (7%) and pyrophyllite (12%) has been treated following Method 1 described under Experimental, using a HNO_3 - $HClO_4$ mixture without HF. The results show that this mixture dissolved only a small proportion of the silicates present (Table 2).

The influence of hydrofluoric acid on the decomposition of this sample has been determined by using different proportions of this acid (0.5–10 ml) with the same concentrations of HNO_3 (1 + 4) and $HClO_4$ (1 + 4). Fig. 1 shows the concentration of aluminium in p.p.m. in solution *versus* the volume of hydrofluoric acid used in the attacks.

The concentration of aluminium in solution increases as the proportion of hydrofluoric acid increases, but above volumes of 4 ml of HF the aluminium concentration remains invariable. The results from 0.5–4 ml give a straight line with correlation coefficient $r = 1.02$ and the equation $y = 11.32x + 95.73$.

These results show that for volumes of 1–4 ml of HF the aluminium in solution comes from the silicate minerals that accompany pyrophyllite, but for volumes greater than 4 ml of HF, the proportion of aluminium in solution does not increase because the pyrophyllite that constitutes the white residue remaining is not decomposed. Attacks on pure pyrophyllite with HNO_3 - $HClO_4$ - HF mixtures show that this mineral is hardly dissolved.

Sample 1 was attacked in the pressure vessel with the same amounts of acid (5 ml of HF) and heated at 50, 100, 125 and 140 °C in order to determine the influence of temperature. The silicon and aluminium liberated are shown in Table 3.

The dissolution of silicon and aluminium increases with temperature, and the sample is completely dissolved when heated at 125 °C not effective when the mineral pyrophyllite is present. However, total decomposition does occur in a pressure vessel at temperatures above 125 °C.

Table 2. Percentages of elements dissolved by HNO_3 - $HClO_4$ attack on sample 3

Element (quoted as oxide)	Total element dissolved, %
Al_2O_3	2.29
Fe_2O_3	0.17
K_2O	0.29
MgO	0.14
CaO	0.23
TiO_2	0

Table 3. Percentages of SiO_2 and Al_2O_3 dissolved by HF in a pressure vessel at various temperatures

Temperature/°C	SiO_2 dissolved, %	Al_2O_3 dissolved, %
50	26.75	20.60
100	41.20	27.59
125	54.85	33.02
140	54.79	33.13

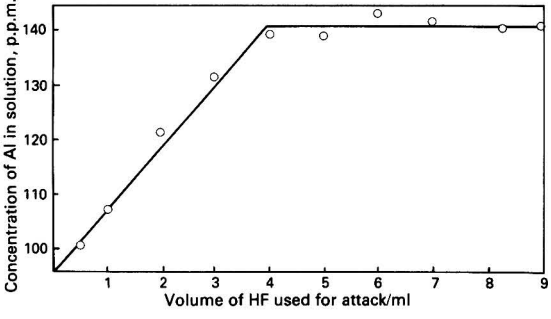

Fig. 1. Concentration of dissolved aluminium in solution *versus* volume of hydrofluoric acid used in the attack

Conclusions

The methods conventionally used to decompose silicate rocks using mixtures of hydrofluoric - perchloric - nitric acid are

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BOOK REVIEWS

Immunoassays in Food Analysis
Edited by B. A. Morris and M. N. Clifford. Pp. xxii + 222. Elsevier. 1985. Price £25. ISBN 0 85334 321 7.

This book is based on the papers presented at the first UK Symposium on Immunoassays in Food Analysis, which was held at the University of Surrey in 1983. It is divided into three sections that cover the principles of immunoassay, its application to the analysis of macromolecules and to the analysis of small molecules.

The first published application of an immunoassay to food analysis appeared in 1970 and in the first chapter M. N. Clifford traces the development of this topic. As an appendix to this chapter, Dr. Clifford has compiled a bibliography of papers (160 papers up to 1983) on food analysis by immunoassay and this provides a most valuable source for the novice in this area. In Chapter 2 (B. A. Morris), the principles and practical aspects of immunoassay development are skilfully expounded. This chapter is packed with useful information on all aspects of assay development (labelling, antibody production, assessment of antisera, assay conditions, separation systems). The coverage is extended to enzyme immunoassay (EIA) in Chapter 3, which includes brief practical details of assay protocols in tabular form. No book on immunoassay would be complete without a chapter on non-isotopic labels, and in Chapter 4 G. W. Aherne surveys fluorescent, chemiluminescent and bioluminescent labels. Time-resolved fluorescence immunoassays and chemiluminescent immunoassay have made considerable progress since 1983, and so this chapter is rather out of date in places but nonetheless provides a useful overview.

The remainder of the book covers specific applications. Food analysis is complicated by the complexity of the sample and the multi-step sample preparation procedures (*e.g.*, meat mixtures must be minced, homogenised, centrifuged and filtered in order to produce a sample suitable for analysis). Various applications are described including EIAs for animal albumins. These are used for species identification of meat (EIA ensures that the meat in your beefburger really is beef and not kangeroo!). Details of assays are also presented for amyloglucosidase in beer (this enzyme is used to produce low-calorie beers) and staphylococcal enterotoxins in food. The third section of the book is devoted to the analysis of small molecules such as ochratoxin A (a toxic secondary metabolite of fungal species such as *Aspergillus*), anabolic hormones (e.g., diethylstilboestrol) and glycoalkaloids (e.g., α-solanine).

Overall this book provides a good introduction to the scope and problems of EIA in food analysis.

L. J. Kricka

Ion Solvation
Yizhak Marcus. Pp. vii + 306. Wiley–Interscience. 1985. Price £42. ISBN 0 471 90756 1.

The author, well known for two earlier works on solution chemistry, "Ion Exchange and Solvent Extraction of Metal Complexes" (with Kertes) in 1969 and "Introduction to Liquid State Chemistry" in 1977, continues this theme with the present volume. Following an introduction, ion solvation in the gas phase, studied largely by mass spectrometry, is briefly dealt with. The author then discusses various interactions that take place during liquid-phase solvation, followed by an analysis of structural and kinetic aspects; here the use of X-ray, infrared and NMR methods makes interesting reading. Chapter Five is devoted to the particular case of ion hydration. Chapters Six and Seven deal with ion - solvent interactions in non-aqueous and mixed solvents, Chapter Eight with ion-pairing reactions (some in molten hydrated salts) and the final chapter discusses applications. The work is interspersed with excellent compilations of relevant data such as appropriate ΔG°, ΔH° and ΔS° values, solvent - ion size parameters and hydration numbers.

This well written and assembled book can be warmly recommended to any chemist concerned with an understanding of ionic reactions in solution, although a good grip on basic thermodynamics would be a prerequisite for a full appreciation of the theory involved. The final chapter on applications where, for example, pH scales in non-aqueous solvents, solvometallurgy, topically interesting nuclear fuel reprocessing reactions, ion-exchange resin behaviour in mixed solvents and relevance to organic synthesis, are discussed, acts as a pleasant leavening of the earlier theoretical rigour.

M. A. Leonard

Spectroscopic Properties of Inorganic and Organometallic Compounds. Volume 17
Senior Reporters G. Davidson and E. A. V. Ebsworth. *Specialist Periodical Report.* Pp. xvi + 395. Royal Society of Chemistry. 1985. Price £95; $138. ISBN 0 85186 153 9; ISSN 0584 8555.

This book is a review of the spectroscopic properties of inorganic and organometallic compounds summarising the chemical literature to the end of 1983. It is produced to the high standard that is normally associated with RSC publications, with clear figures and chemical structures. The book is sub-divided on a technique basis into seven chapters by different authors, *viz.*, NMR by B. E. Mann with 2856 references in 154 pages; NQR by K. B. Dillon with 96 references in 17 pages; Rotational Spectroscopy by S. Cradock with 105 references in 10 pages; Characteristic Vibrations of Main-group Elements by S. Cradock with 344 references in 21 pages; Vibrational Spectra of Transition-element Compounds by G. Davidson with 350 references in 27 pages; Vibrational Spectra of Some Co-ordinated Ligands by G. Davidson with 426 references in 51 pages; Mössbauer Spectroscopy by J. D. Donaldson, S. J. Clark and S. M. Grimes with 771 references in 94 pages; and Gas-phase Molecular Structures Determined by Electron Diffraction by D. W. Rankin and H. E. Robertson with 59 references in 14 pages.

The different authors have adopted a similar format for each chapter. Within each chapter the work is summarised on a problem rather than technique basis. Key reviews in each area are cited together with a comprehensive overview of the literature. The various authors have succeeded in the gigantic task of condensing information from over 5000 references into a book of this size (395 pages).

The major criticism of the book is that the inevitable time lag in producing the book, almost 2 years from the period covered to publication, reduces its impact on specialists working in the various areas. The Editor promises that the next volume will be produced from camera-ready copy, which should reduce this time.

In summary, this is an excellent book, which provides a good overview of the diverse spectroscopic techniques used to study inorganic and organometallic compounds. It will be a useful reference manual for specialists and others interested in this subject.

D. P. Leworthy

Trace Analysis. Volume 4
Edited by James F. Lawrence. Pp. xii + 305. Academic Press. 1985. Price $65; £65. ISBN 0 12 682104 6.

This is the second volume of the Trace Analysis series that I have received, the previous one being Volume 3. Although I found this volume less stimulating than Volume 3, it was equally well presented and provides a mine of useful information concerning the topics covered.

Chapter 1, concerning ion-selective membrane electrodes and written by Les Ebdon and Beverley King, is well written and covers most aspects of the design and application of ion-selective electrodes. It would have been of benefit, however, to have had some comparisons between the enzyme and immuno-electrodes presented with other non-potentiometric electrochemical sensors.

The second review, by Terry F. Bidleman, on high-volume collection of organic vapours using solid absorbants, is a workmanlike presentation with comprehensive lists of materials used to collect pollutants and adequate descriptions of methods for collection. The third review, on "Trace Analysis of Environmental Samples by X-ray Emission Spectroscopy," is an excellent guide to the technique with a good and easily understandable explanation of X-ray techniques and their application to air and water pollution analysis.

The fourth review, "Inductively Coupled Plasma - Atomic Emission Spectrometry Applied to Elemental Analysis," was similarly presented but the authors, John R. Garbarino and Howard E. Taylor from the US Geological Survey, seem not to have noticed that major developments in this field have occurred outside the USA. Thus the references cited are predominantly those published in the USA. Either these authors are not aware of the source of some of the developments they quote, for example, electrochemical vaporisation into the ICP, or they suffer from the "not invented here" syndrome.

The final review, "Trace Analysis of Wet Atmospheric Deposition by Nuclear Methods" (Landsberger, Jervis and Monaro), is also well presented and describes theoretical and practical considerations of neutron activation analysis and proton-induced X-ray emission (PIXE) in addition to the analytical procedures, constraints and interpretation of the method. In their conclusions, these authors present a real assessment of the techniques and offer areas for improvement rather than claim the techniques to be ultimate. To quote them, "certainly the last chapter on the analysis of atmospheric precipitation chemistry has not been written yet." This is also true of most analytical techniques and I look forward to reading subsequent volumes of Trace Analysis series to find out more.

R. D. Snook

Synthetic Polymeric Membranes. A Structural Perspective. Second Edition
Robert E. Kesting. Pp. xiv + 348. Wiley-Interscience. 1985. Price £55.75. ISBN 0 471 807 17 6.

This book is devoted to thin polymer films, as solids or liquids, which act as semipermeable barriers for permeants in the gaseous, liquid or solid state. It ought, therefore, to be of interest to scientists of various specialisations, including analytical scientists. It is towards the last named group that this review is directed, and who will be concerned with polymer filtration dialysis, gas separations, membrane electrodes, ion-exchange gel permeation chromatography, etc.

A scan of the chapter headings reveals that there is not a great deal for the routine analyst, who will in any case be using well tried systems. Thus, out of the ten chapters devoted to topics such as biological membranes, liquid membranes, porous membranes, phase inversion membranes, dense membrane, polymer solutions and membrane polymers, it is only those devoted to membrane separation processes and miscellaneous uses of membranes that will be of major interest. Nevertheless, the three chapters covering membrane polymers, separations and miscellaneous uses make up just over half the book. Even so, the treatment is frequently cursory; for example, five pages can hardly do justice to membranes for electrodes.

Having regard to emphasis and price, this cannot be recommended for the wider readership, although there are points of general interest to the more forward looking research analyst through access to libraries.

J. D. R. Thomas

Plasma Chromatography
Edited by Timothy W. Carr. Pp. xiv + 259. Plenum. 1984. Price $37.50. ISBN 0 306 41432 5.

Plasma chromatography, an emerging analytical technique, is based on the principle of the separation of ion-molecules produced from a sample gas containing organic molecules contacted with ions to convert each organic molecule into a very stable ion-molecule. The ion-molecules are separated by injection into a tube of non-reactive gas through which they are progressed by a strong electric field to arrive at a collector as ion peaks at times related to their structure. The first patent for the technique was applied for in 1968 and issued in 1972. This book is devoted to progress made.

The objective has been to deal in the first part of the book with fundamental aspects covering instrument design (G. E. Spangler and M. J. Cohen), ion mobility theory (E. A. Mason) and theory of atmospheric pressure ionisation (M. W. Siegel). This plan has led to a sound introduction to all the essentials of basic instrument design and performance, sampling handling considerations, use of the plasma chromatograph as a gas chromatographic detector and all the basic theory and rate equations for reagent ion and sample ion production and for ion depletion. The result is a readable and easy to follow account supported by well drawn and clear diagrams and systematically developed equations.

Four of the remaining chapters are devoted to applications with well chosen underlying theory for covering the behaviour of isomeric compounds for illustrating the use of ion mobility spectra in analysis (D. F. Hagen), use of plasma chromatography as a gas chromatographic detector (H. H. Hill and M. A. Bain) and applications in the areas of toxic airborne chemical analysis (R. J. Dam) and analysis of semiconductor surfaces and gases inside microelectronic packages (T. W. Carr).

The final chapter, devoted to instrument design and automation (R. F. Wernlund). is an appropriate ending for stimulating the reader on to more elaborate possibilities, such as coupling with mass spectrometry and computer interfacing.

The book emphasises that plasma chromatography is an extremely sensitive detection technique. It also discusses the other advantages, such as information about the size of molecular species, but with cautious reminders of limitations and the need for attention to such matters as the internal standardisation of the instrumental technique. All this points to a good book on the pioneering of plasma chromatography, which is an appropriate introduction to all who wish to learn something of the technique.

J. D. R. Thomas

Advances in Steroid Analysis '84
Edited by S. Görög. *Analytical Symposia Series, Volume 23.* Pp. xii + 604. Elsevier. 1985. Price $139; Dfl375. ISBN 0 444 99533 1.

I recently organised a members' papers meeting of my Association and was concerned that I was not able to check the contents of the talk in the same way that I would if the paper had been submitted for publication in a journal. In the event, the papers were very good and this is also the case with this book, which reports the papers presented at the 1984 Szeged (Hungary) Symposium. The book contains 66 papers presented by steroid analysts working in the field with a bias towards the methodology of steroid analysis. The range of steroids considered ranges across all of the important groups including hormones, both bound, total and free, vitamins, cardiac drugs and bile salts. The range of methods covered represents the latest technology such as HPLC, GC, RIA, EIA and densitometry. My particular subjects of interest included "blood spot levels of 17-hydroxyprogesterone on CAH" and "steroid levels during sports performance."

Billed as an International Forum on Steroid Analysis, it should be recognised that most of the papers presented are from Eastern Bloc countries, although there are some papers from the UK (Steroid analysis in saliva from the Welsh National School of Medicine and Sterols by GC - MS from Glasgow Royal Infirmary).

The fact that this is the 23rd in the series speaks for the success of this type of book but, because of the wide-ranging nature of the topics, it is difficult to visualise it as a textbook for everyone but more as a book that should be on the shelf of the routine and research laboratory in order to convey the state of the art in steroid analysis and to avoid duplication in research programmes.

J. F. Stevens

Cosmetic Analysis. Selective Methods and Techniques
Edited by P. Boré, *Cosmetic Science and Technology Series, Volume 4.* Pp. xii + 534. Marcel Dekker. 1985. Price $85 (US and Canada); $102 (Rest of World). ISBN 0 8247 7113 3.

Before deciding whether to purchase this expensive volume, analysts must assess how much of it will be of direct use to them. The Editor and the authors all come from L'Oréal, France, and consequently the approach is that of a large manufacturer's laboratories. The chapters vary between techniques and classes of materials and of the nine chapters only five would be of some help to a control laboratory in checking compliance to the EEC Cosmetic Products Directive.

The opening chapter discusses the spectral analysis of polymers used in hair and nail cosmetics, for which detailed separation schemes are given with guidance on the characteristics of IR and ^{1}H and ^{13}C NMR spectra. Further details of these spectra are gathered in an enormous 231-page Appendix, which illustrates 112 spectra, and whilst this Appendix unbalances the book, the information may well be of use in areas outside cosmetics.

A brief account of voltammetry follows for three specific applications. Polarographic methods are all too often dismissed, but I doubt if many will be persuaded to buy the equipment on the strength of a few examples. Another short chapter follows on the determination of mercaptoacetic acid, but surprisingly no reference is made to the similar official EEC method.

The fourth chapter is a clear account of the application of pyrolysis GC to characterising and identifying high polymers. The same technique is then discussed in relation to quaternary ammonium compounds. A further technique, headspace analysis, is reported for three particular determinations of residues, *viz.*, solvents and monomers in polymers, 1,4-dioxane in surfactants and hydrogen sulphide.

One of the major analytical problems for cosmetic products is the identification and determination of preservatives, of which about 60 may be used. The seventh chapter is a comprehensive review of nine TLC systems that can be combined with nine spray reagents to aid identification. This is information that will remain of use even after an official EEC method is published, but it is disappointing that nothing is offered for the quantitation of preservatives.

The penultimate chapter is a short discussion of ion-exchange chromatography as applied to the analysis of protein derivatives and amino acids. The last chapter is on the analysis of oxidation hair dyes by HPLC and incorporated within its compass there is a fine overview of the technique.

This book will be of clear usefulness to other manufacturing laboratories, but of considerably less relevance to enforcement laboratories. The volume abounds in tables and figures and the printing and binding are of high quality, but the number of minor errors is annoyingly high for such a high-priced book.

S. Crisp

Ion- and Molecule-selective Electrodes in Biological Systems
J. Havas. Pp. 238. Springer-Verlag. 1985. Price DM98. ISBN 3 540 13725 4.

This is a tightly packed, informative book, written by an author who has been closely connected with electrochemical measurements in the biological and general scientific field since about the time of the burst of interest in analytical potentiometry occasioned by the ion-selective electrode developments of the 1960s. The main subject matter is very usefully introduced by the opening chapter on equilibria in biological fluids but, except for one page, this is devoted to blood fluids. The neglect of the other fluids is surprising in view of the importance of electrochemical measurements in sweat, saliva, urine, etc., and having regard to the fact that many such measurements are discussed in the later chapters. Nevertheless, the discussion on blood fluids is well done, with particular and proper emphasis to acid - base equilibria.

The next three chapters are concerned, respectively, with methodology and instrumentation (Chapter 2), ion-selective electrode applications, which include pH, sodium, potassium, calcium, chloride and fluoride electrodes, and which, essentially, account for just over a third of the subject matter of the book (Chapter 3), and molecule-selective electrode applications devoted to oxygen, hydrogen, carbon dioxide, ammonia and enzyme systems (Chapter 4). There is a final chapter of five pages on ion-selective field effect transistors which, in view of recent rapid developments, is far from up-to-date. Indeed, it is in this last respect that the whole book can be faulted for there are but few references to work published since 1979, although there are one or two references for 1983.

The book is imaginative in mode of presentation with clearly presented and well illustrated text preceding the very useful summary tables of applications of the various types of electrodes and sensors which, occasionally, go beyond potentiometric sensors to coulometric and amperometric systems. If the tables were up to date, the book would be a sell-out, as the brief summary relating to each application area is a model of

conciseness in summarising essential information. For example, for a potassium determination it is deduced at a glance that a flow-through electrode was used on whole blood in open-heart surgery and that the potassium values agreed with flame photometry. The reference to each application is keyed to the reference list at the end of the book for the reader who needs source publication.

There are a few unfortunate statements, such as the total concentration of ions (anions and cations) in blood fluid being *ca.* 150 mmol dm^{-3}, and some misprints. On the credit side is a discussion of safety considerations underlying the use of electrical instrumentation during *in vivo* monitoring, a good index and an extensive reference list, even if not recent. On the whole, this is a sufficiently different book from others in the field for it to be recommended for purchase.

J. D. R. Thomas

Biological Reference Materials: Availability, Uses and Need for Validation of Nutrient Measurement
Edited by Wayne R. Wolf. Pp. xviii + 425. Wiley–Interscience. 1984. Price £69.40. ISBN 0 471 80636 6.

This hardback, printed from typescripts, is sub-titled "Availability, Uses and Need for Validation of Nutrient Measurement." However, its coverage includes environmental and clinical materials. It consists of the proceedings, forming about 80% of the volume, of a meeting on biological reference materials held in 1983, and an Appendix that reproduces *in toto* a 1982 publication from the US National Bureau of Standards (NBS) of the proceedings of a workshop held in 1980 on reference materials for organic nutrient measurement.

The main proceedings consist of 17 contributions, arranged in groups, by 26 participants of whom 21 hail from North America. The first section of six papers covers programmes for certified reference materials, available or in preparation, of bodies such as the NBS, the EEC Bureau of Reference and the International Atomic Energy Agency. Some analytical information is provided on existing materials and needs for other materials are assessed. Most of the analytes are trace elements but organic constituents of clinical reference materials and edible oils are also mentioned. The matrices range from marine sediments through aquatic and land plants to sewage sludge and animal tissues.

Emphasis is placed on requirements for reference materials, such as homogeneity and stability, in the second section of four papers, which covers the problems of obtaining and characterising materials and of spiking materials to obtain adequate levels of analytes of interest. Three of the four papers in Part 3 are concerned with the provision and use of quality control materials for nutrient (food) analysis and the fourth is an interesting examination of the use of analyte pairs in a reference material as a means of checking analyte dilution. The final two papers address problems of environmental specimen banking: the first considers requirements for valid sampling and the second reviews trace metal analysis, largely by atomic absorption, in a variety of materials within the programme for the German Environmental Specimen Bank.

At the end of the formal presentations, an open discussion forum was held in which two key themes of the role of specimen banking and the needs, production, uses and coordination of biological reference materials were explored. Apart from the introductory remarks and a summary, the discussion reported for each topic unfortunately extends to less than one page and is of limited value.

The 1980 workshop, reported in the Appendix, was concerned with the then current state of measurement techniques, with matrices suitable for reference materials, and considered especially fats, including cholesterol, vitamins and sugars. It made several recommendations and suggested further needs in the NBS programme.

This book will be useful to someone new to this specialised and important area and may be of value to groups already active in the fields covered. It is a useful source of information otherwise difficult to gather, including helpful background material, and has a comprehensive, although terse, index. However, its price and the inclusion of the NBS report, published previously and occupying nearly one quarter of the contents, will restrict its appeal.

D. H. Calam

A001 for further information. See page iv.

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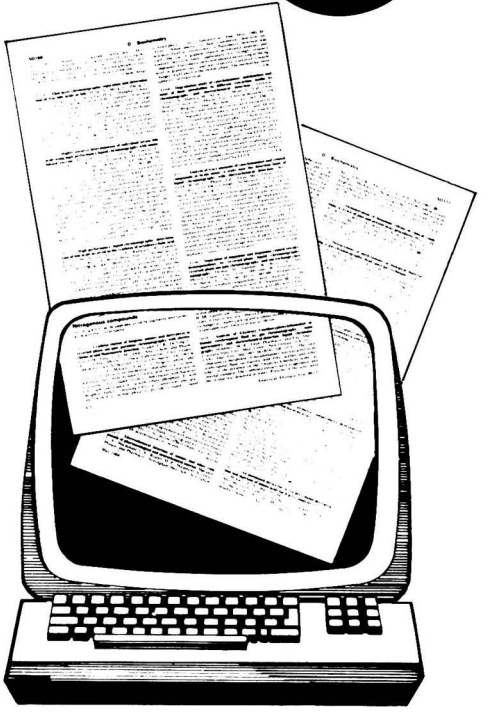

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