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# Determination of Hydroquinone in Skin-toning Creams Using High-performance Liquid Chromatography

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A simple, rapid reversed-phase HPLC method for the determination of hydroquinone in skin-toning creams is described that is suitable for routine use. The sample is dissolved in methanol or methanol - light petroleum and directly injected without further purification. The method successfully passed a ruggedness test and was applied to a range of 35 creams.

**Keywords:** *Hydroquinone determination; high-performance liquid chromatography; skin-toning creams; cosmetic product*

The major user of skin-toning creams in the UK is the West Indian population. The creams are applied to even out the skin colour on the facial areas. They are believed to function by decolourising the melanin in the skin and preventing new melanin being formed. The most favoured material for use in skin-toning preparations is hydroquinone, which was reported by Spencer[1] to be effective at 1.5–2% in a vanishing cream producing a temporary lightening of skin colour. Spencer found that a concentration of 5% was liable to cause redness and burning. Cases of patchy de-pigmentation have arisen following the use of some skin-toning creams available on the retail market.[2]

Council Directive 76/768/EEC of the European Communities makes the general point that cosmetic products must not be harmful under normal or foreseeable conditions of use and specifically allows hydroquinone to be used in cosmetic products at a level of 2% *m/m* subject to certain conditions of use and warnings that must be printed on the label. The field of application is not specified in the basic Directive 76/768/EEC.[3] The second amendment, Council Directive 82/368/EEC,[4] specified the field of application as oxidising colouring agents for hair dyeing and excluded hydroquinone for use as a skin lightener from the scope of the Directive. The fifth Commission Directive 84/415/EEC[5] permitted the use of hydroquinone as a localised skin-lightening agent subject to a maximum concentration of 2% *m/m* in the finished cosmetic product and a warning on the label containing the information "contains hydroquinone, avoid contact with the eyes, apply to small areas, if irritation develops discontinue use, do not use on children under the age of 12."

Member States were asked to bring into force the laws, regulations and administrative provisions necessary to comply with the Directive by not later than 31st December 1985. The method described in this paper will be submitted to the EEC for consideration as the adopted method of analysis. Hydroquinone is permitted at a concentration of up to 2% by mass under the Cosmetic Products (Safety) Regulations; Statutory Instrument 1984: No. 1260, which had to be complied with by 1st January 1986. However, recent studies have shown that some available products contain more than the permitted level of hydroquinone.[6]

In the light of these facts it was decided to carry out an extensive survey of skin-toning creams to determine their hydroquinone content.

There is a dearth of literature on the determination of hydroquinone in skin-toning creams. The most recent published work of Popov and Yanishlieva[7] involved extraction of hydroquinone with acetic acid and conversion of the hydroquinone into *p*-benzoquinone, with subsequent spectrophotometric determination. The determination of hydroquinone in various sample matrices has been described although none included skin-toning creams.[8–11] The method described here is of wide application and is rapid, allowing ten samples and associated standards to be analysed in less than 3 h.



## Experimental

### Apparatus

Reversed-phase HPLC was performed at ambient temperatures using a Spectrophysics SP800 solvent delivery system and a Shimadzu SPD-MIA diode-array UV - visible spectrophotometric detector.

### Reagents

All reagents were of analytical-reagent grade. The methanol was of solvent for liquid chromatography grade.

### Chromatographic Conditions

The analytical column used was of stainless steel (250 mm × 4.6 mm i.d.) packed with Spherisorb S5-ODS of 5 μm. The sample injection volume was 10 μl. The mobile phase was methanol - water (10 + 90 *V/V*) pumped at a flow-rate of 1.5 ml $min^{-1}$. The detector was operated at 226 nm with a sensitivity of 0.50 A full scale and a chart speed of 5 mm $min^{-1}$.

### Skin-toning Cream Samples

Samples of cream were bought from a number of retail outlets in the South London area. They were stored at room temperature throughout the investigation.

### Procedure

*Extraction of hydroquinone*

Transfer 0.05 g of cream containing 0.2–4.0% of hydroquinone into a 10-ml calibrated flask and add 8.0 ml of methanol. Heat to 40 °C in a water-bath and shake occasionally until dissolved. Allow to cool and make up to the mark with methanol. If the cream fails to dissolve under these conditions repeat the procedure with 4.0 ml of light petroleum (60–80 °C boiling range) and make up to the mark with methanol.

**Table 1.** Multi-factorial experiments for the ruggedness test

| Factor | Experiment 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| *A/a* | *A* | *A* | *A* | *A* | *a* | *a* | *a* | *a* |
| *B/b* | *B* | *B* | *b* | *b* | *B* | *B* | *b* | *b* |
| *C/c* | *C* | *c* | *C* | *c* | *C* | *c* | *C* | *c* |
| *D/d* | *D* | *D* | *d* | *d* | *d* | *d* | *D* | *D* |
| *E/e* | *E* | *e* | *E* | *e* | *e* | *E* | *e* | *E* |
| *F/f* | *F* | *f* | *f* | *F* | *F* | *f* | *f* | *F* |
| *G/g* | *G* | *g* | *g* | *G* | *g* | *G* | *G* | *g* |
| Results | *s* | *t* | *u* | *v* | *w* | *x* | *y* | *z* |
| Hydroquinone, % | 2.062 | 2.045 | 2.127 | 2.020 | 2.086 | 2.070 | 2.100 | 2.056 |

| | Factor *A* | *a* |
|---|---|---|
| Wavelength of measurement/nm | 226 | 230 |
| | *B* | *b* |
| Mass of cream taken for analysis/g | 0.05 | 0.08 |
| | *C* | *c* |
| Detector type | Shimadzu SPD-MIA | Pye Unicam PU4020 |
| | *D* | *d* |
| Column length/mm | 250 | 100 |
| Internal diameter/mm | 4.6 | 3 |
| Packing material | Spherisorb S5-0DS | Cp-$t_m$-Spher $C_{18}$ |
| Constructional material | Stainless steel | Glass |
| | *E* | *e* |
| Mobile phase flow-rate/ml $min^{-1}$ | 1.5 | 2.0 |
| | *F* | *f* |
| Final volume of extracting solution/ml | 10 | 25 |
| | *G* | *g* |
| Mobile phase composition (methanol - water) | 10 + 90 | 12.5 + 87.5 |

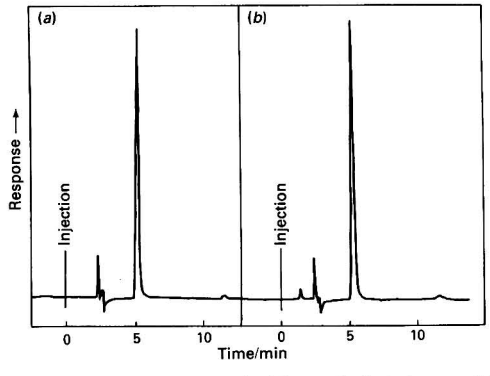


**Fig. 1.** Typical chromatograms for (*a*) a standard solution containing 0.152 g $l^{-1}$ of hydroquinone and (*b*) a skin-toning cream containing 1.6494% *m/m* of hydroquinone

*Preparation of the stock standard solution*

Prepare a stock solution of standard hydroquinone by dissolving the solid hydroquinone in methanol at a concentration of 10 g $l^{-1}$.

*Preparation of the calibration graph*

Prepare a range of solutions by diluting aliquots of the stock hydroquinone standard with methanol to 100 ml in calibrated flasks. Inject the standard solutions and measure the peak-height absorbance. A straight-line calibration graph of absorbance *versus* concentration was obtained which passed through the origin.

**Table 2.** Effect of each factor in the ruggedness test

$D_a = 1/4\,(s + t + u + v - w - x - y - z) = 0.0145$
$D_b = 1/4\,(s + t - u - v + w + x - y - z) = 0.0100$
$D_c = 1/4\,(s - t + u - v + w - x + y - z) = 0.0460$
$D_d = 1/4\,(s + t - u - v - w - x + y + z) = 0.0100$
$D_e = 1/4\,(s - t + u - v - w + x - y + z) = 0.0160$
$D_f = 1/4\,(s - t - u + v + w - x - y + z) = 0.0295$
$D_g = 1/4\,(s - t - u + v - w + x + y - z) = 0.0155$

## Results and Discussion

Under the experimental conditions used, hydroquinone had a retention time of 5.0 min. Fig. 1(*a*) and (*b*) depict typical chromatograms of a standard solution and skin-toning cream. It can be seen that there is no overlap from other compounds present in the cream as these are not eluted by the relatively weak mobile phase composition employed in the method.

### Performance Characteristics

The limit of detection, which was based on a solution containing 0.001 g $l^{-1}$ of hydroquinone and defined as 5 times the standard deviation of this standard, was found to be 0.13% *m/m* with 4 degrees of freedom. A skin-toning cream containing 2% *m/m* of hydroquinone produces a solution that gives an absorbance of approximately 0.17. A proprietary skin-toning cream containing 1.694% *m/m* of hydroquinone and spiked with 2.000% *m/m* of hydroquinone gave a recovery of 3.658 ± 0.232% *m/m* (95% confidence limits, 4 degrees of freedom). A proprietary moisturising cream base spiked with 2.000% *m/m* of hydroquinone gave a recovery of 2.045 ± 0.0186% *m/m* (95% confidence limits, 4 degrees of freedom). The full analytical procedure was followed through for five

**Table 3.** Results for a range of skin-toning creams

| Sample No. | Test No. | Hydroquinone, % | Sample No. | Test No. | Hydroquinone, % |
|---|---|---|---|---|---|
| 1 | 1 | 5.40 | 19 | 1 | 2.07 |
| | 2 | 5.69 | | 2 | 1.89 |
| 2 | 1 | 8.30 | 20 | 1 | 1.62 |
| | 2 | 8.07 | | 2 | 1.76 |
| 3 | 1 | 2.25 | 21 | 1 | 2.08 |
| | 2 | 2.20 | | 2 | 1.99 |
| 4 | 1 | 1.94 | 22 | 1 | 1.76 |
| | 2 | 1.86 | | 2 | 1.74 |
| 5 | 1 | 2.09 | 23 | 1 | 1.94 |
| | 2 | 2.08 | | 2 | 1.95 |
| 6 | 1 | 5.99 | 24 | 1 | 1.62 |
| | 2 | 6.28 | | 2 | 1.64 |
| 7 | 1 | 5.55 | 25 | 1 | 2.37 |
| | 2 | 5.41 | | 2 | 2.49 |
| 8 | 1 | 1.90 | 26 | 1 | 6.44 |
| | 2 | 2.01 | | 2 | 6.63 |
| 9 | 1 | 1.75 | 27 | 1 | 1.72 |
| | 2 | 1.60 | | 2 | 1.75 |
| 10 | 1 | 1.74 | 28 | 1 | 1.64 |
| | 2 | 1.61 | | 2 | 1.71 |
| 11 | 1 | 1.88 | 29 | 1 | 1.66 |
| | 2 | 1.87 | | 2 | 1.64 |
| 12 | 1 | 1.96 | 30 | 1 | 1.98 |
| | 2 | 1.90 | | 2 | 2.07 |
| 13 | 1 | 5.20 | 31 | 1 | 1.79 |
| | 2 | 5.08 | | 2 | 1.76 |
| 14 | 1 | 2.06 | 32 | 1 | 1.80 |
| | 2 | 1.93 | | 2 | 1.76 |
| 15 | 1 | 2.37 | 33 | 1 | 2.18 |
| | 2 | 2.24 | | 2 | 2.18 |
| 16 | 1 | 2.11 | 34 | 1 | 2.03 |
| | 2 | 2.20 | | 2 | 2.11 |
| 17 | 1 | 5.10 | 35 | 1 | 5.36 |
| | 2 | 4.86 | | 2 | 5.19 |
| 18 | 1 | 1.52 | | | |
| | 2 | 1.56 | | | |

samples of the same tube of skin-toning cream; a mean of 1.694% *m/m* and standard deviation of 0.017% *m/m* of hydroquinone were found. Replicate injections of standard solutions of hydroquinone of concentrations of 0.010 and 0.200 g $l^{-1}$ gave standard deviations of 0.000299 and 0.00217, respectively (4 degrees of freedom), which is the same order of magnitude to that obtained with skin-toning creams, indicating that the extraction procedure does not contribute significantly to the over-all error of the procedure.

### Ruggedness Test

The results presented so far indicate that the method is precise and without significant bias. However, these results were obtained by a single analyst using a rigidly defined set of operating conditions in one laboratory. It was thought desirable to simulate use of the method in other laboratories by altering slightly the various analytical parameters and determining the effect on the result. The Youden and Steiner[12] model was employed for this test. The ruggedness test is carried out by deliberately varying the factors that are likely to have an effect on the result, from value $A$ to value $a$. Youden and Steiner give a set of multi-factorial experiments for varying up to seven factors simultaneously (Table 1). From these eight results the effect of each factor can be calculated for a proprietary skin-toning cream (Table 2). The standard deviation ($\sigma$) of the eight individual results found on this occasion was 0.0334. Any value of $D$ can be considered significant ($P<0.05$) if $|D| > \sqrt{2}\sigma$, ($\sqrt{2}\sigma = 0.0472$).

As no value of $D$ exceeds $\sqrt{2}\sigma$ the method can be considered to be rugged for the parameters chosen.

### Application to Samples

The method was applied to a range of skin-toning creams. Four products were waxy in nature and required preliminary dissolution in light petroleum before addition of methanol. Each sample was analysed in duplicate and the results are given in Table 3. Eight products contained hydroquinone well in excess of the 2% *m/m* permitted by the Cosmetic Products (Safety) Regulations, 1984.

In order to obtain some indication of the relative errors of the procedure the difference between the duplicate results was plotted against the mean value for each sample. This plot revealed two distinct clusters at about 2% *m/m* and 5.5% *m/m* of hydroquinone. The ratio of the mean of the differences to the mean of the sample means was taken for each cluster and was found to be very similar at the two hydroquinone levels (0.0368 at 2% hydroquinone, 0.0371 at 5.5% hydroquinone) indicating that the relative errors of the procedure are not dependent on hydroquinone concentration in the sample.

The method presented here allows the determination of hydroquinone in a complex sample matrix with relative ease, accuracy and precision and has been shown to be insensitive to changes in many instrumental parameters, permitting its application in a wide range of laboratories.

## References

1. Spencer, M. C., *Arch. Dermatol.*, 1961, **84**, 131.
2. Ridley, C. M., *Br. Med. J.*, 1984, **287**, 1537.
3. Council Directive 76/768/EEC, *Off. J. Eur. Commun.*, 1976, L262.

4. Second Amendment Council Directive 82/368/EEC, *Off. J. Eur. Commun.*, 1982, L167.
5. Fifth Amendment Commission Directive 84/415/EEC, *Off. J. Eur. Commun.*, 1984, L228.
6. Bush, S. J., personal communication, 9 November 1984.
7. Popov, A., and Yanishlieva, N., *Fresenius Z. Anal. Chem.*, 1970, **249**, 191.
8. Miller, R. L., *Chromatogr. Newsl.*, 1981, **9**, 10.
9. Greenlee, W. F., Chisn, J. P., and Richert, D. E., *Anal. Biochem.*, 1981, **112**, 367.
10. Sticher, O., Soldati, F., and Lehmann, D., *Plant. Med.*, 1979, **35**, 253.
11. Raghavan, N. V., *J. Chromatogr.*, 1979, **168**, 523.
12. Youden, W. J., and Steiner, E. H., "Statistical Manual of the Association of Official Analytical Chemists," AOAC, Washington, DC, 1975.

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# Stability-indicating Assay for Oxyphenbutazone

## Part II.* High-performance Liquid Chromatographic Determination of Oxyphenbutazone and Its Degradation Products

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A high-performance liquid chromatographic method is proposed for the simultaneous determination of oxyphenbutazone and six potential decomposition products, using a reversed-phase column and ultraviolet detection. The method is more sensitive than thin-layer chromatography and allows the determination of 0.1% of each degradation product (with respect to oxyphenbutazone). It has been applied to the analysis of commercial tablets, capsules and ointments.

**Keywords**: *Oxyphenbutazone determination; degradation products determination; high-performance liquid chromatography; stability-indicating assay; reversed-phase chromatography*

In Part I,[1] we outlined the difficulties relating to the establishment of a thin-layer chromatographic (TLC) method to be used as a stability-indicating assay of oxyphenbutazone. We proposed a quantitative thin-layer chromatographic procedure that prevents air oxidation on the plate of oxyphenbutazone.

As reversed-phase high-performance liquid chromatography (HPLC) is particularly suitable for the determination of easily oxidised compounds, we propose here a reversed-phase HPLC procedure for separating and determining oxyphenbutazone and six potential decomposition products.

### Method Development

Oxyphenbutazone and its potential decomposition products were chromatographed under different conditions in order to optimise the separation. The decomposition products, the formulae of which were given in Part I,[1] were 4-hydroxy-4-butyl-1-phenyl-2-(4-hydroxy)phenylpyrazolidine-3,5-dione (**I**), 2-butyl-*N*-(4-hydroxyphenyl)-*N'*-phenylpropanediamide (**II**), 2-[1-phenyl-2-(4-hydroxy)phenylhydrazino]-3-oxo-2-butylpropionic acid (**III**), 4-hydroperoxy-4-butyl-1-phenyl-2-(4-hydroxyphenyl)pyrazolidine-3,5-dione (**IV**), 2-butyl-*N*-{3-[4-butyl-3,5-dioxo-1-(4-hydroxyphenyl)-2-phenylpyrazolidin-4-yl]-4-hydroxyphenyl}-*N'*-phenylpropanediamide (**V**) and 2-oxo-3-butyl-3-phenylcarbamoyl-5-hydroxyindole (**VI**). The starting solvent system was the mobile phase we previously used in the stability-indicating assay of phenylbutazone, *viz.*, 0.1 M tromethamine (THAM) citrate buffer (pH 5.25) - acetonitrile (60 + 40).[2] As this mobile phase could not achieve the separation of the compounds, the influence of pH and acetonitrile content of the mobile phase on the capacity factor was investigated. The method was developed using a mixed standard solution (5 μg ml$^{-1}$) of each compound (Fig. 1). The capacity factor, $k'$, was calculated using the equation $k' = (u/L)(t_R + 1)$, where $t_R$ is the retention time of the compound, $u$ the linear flow-rate of the mobile phase given by the supplier and $L$ the length of the column.

As a stability-indicating assay of oxyphenbutazone in drugs involves the determination of less than 1% of each decomposition product (relative to the drug), the resolution factor (R.F.) between oxyphenbutazone and **VI** (pH 4.1) and oxyphenbutazone and **I** (pH 5.25 and 6.4) was calculated using the equation R.F. $= 2(t_{R_2} - t_{R_1})/(w_2 + w_1)$, where $t_{R_1}$ and $t_{R_2}$ are the retention times and $w_1$ and $w_2$ the peak widths. The influence of the acetonitrile content of the mobile phase on the resolution factor is shown in Fig. 2.

The pH values 5.25 and 6.4 gave a satisfactory resolution (R.F. > 2 with equal concentrations of each compound) but pH 6.4 was discarded because of the elution of compound **III** [Fig. 1(*a*)] in the solvent peak (the mobile phase cannot be used as the solvent because of the instability of the compounds in the solvent system). Therefore, the mobile phase 0.1 M THAM citrate buffer (pH 5.25) - acetonitrile (65 + 35) was selected in further development of the method. In order to increase the resolution, the effect of the addition of tetrahydrofuran (THF) (2–6%) on this mobile phase was investigated. The addition of 6% of THF increased the selectivity and gave a resolution factor R.F. = 1.32 between oxyphenbutazone and **I** using a concentration of 500 μg ml$^{-1}$ of oxyphenbutazone and 5 μg ml$^{-1}$ of **I**.

The mobile phase A finally selected was 0.1 M THAM citrate buffer (pH 5.25) - acetonitrile - THF (65 + 29 + 6).

For the determination of **V** a more strongly eluting mobile phase B, 0.1 M THAM citrate buffer (pH 5.25) - acetonitrile (45 + 55), was selected from Fig. 1(*b*).

### Experimental

#### Apparatus

A high-performance liquid chromatograph (Merck LMC System), equipped with a variable-wavelength ultraviolet detector (LC 313), a 10-μl loop injector and fitted with a 25 × 0.4 cm i.d. stainless-steel cartridge, packed with 7 μm LiChrosorb RP-18 (Merck), was used. The column was equilibrated with the mobile phase for 30 min before use.

#### Reagents and Materials

Tromethamine and citric acid were of analytical-reagent grade. Oxyphenbutazone and its degradation products (**I–VI**) were the same as used previously.[1]

Tanderil ointment (5% oxyphenbutazone), Tanderil tablets (100 mg of oxyphenbutazone per tablet) were commercial formulations (Ciba-Geigy Laboratories). Kymalzone capsules (75 mg of oxyphenbutazone per tablet) were commercial formulations (Biocodex Laboratories).

* For Part I of this series, see reference 1.

† To whom correspondence should be addressed.

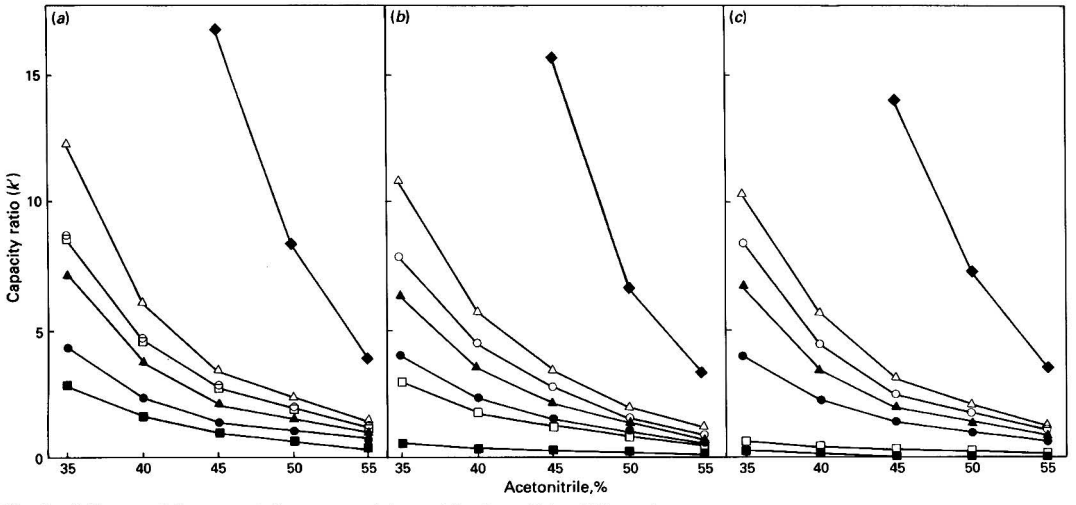


**Fig. 1.** Influence of the acetonitrile content of the mobile phase (0.1 M THAM citrate buffer - acetonitrile) on the capacity factors ($k'$) of (□) oxyphenbutazone and its degradation products: (●) **I**, (△) **II**, (■) **III**, (▲), **IV**, (◆) **V** and (○) **VI**. (*a*) Buffer pH 4.10; (*b*) buffer pH 5.25; and (*c*) buffer pH 6.40

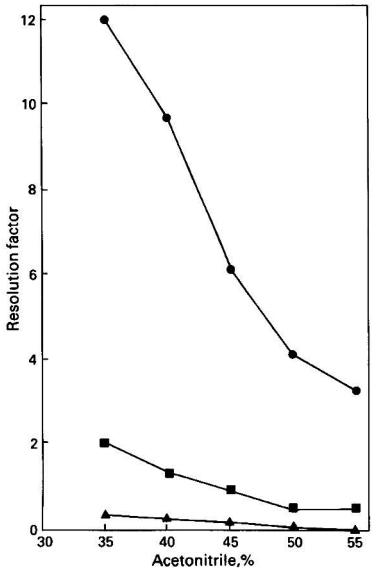


**Fig. 2.** Resolution factor *versus* the acetonitrile content of the mobile phase (0.1 M THAM citrate buffer - acetonitrile): (▲) between oxyphenbutazone and **VI**, buffer pH 4.10; (■) between oxyphenbutazone and **I**, buffer pH 5.25; and (●) between oxyphenbutazone and **I**, buffer pH 6.40

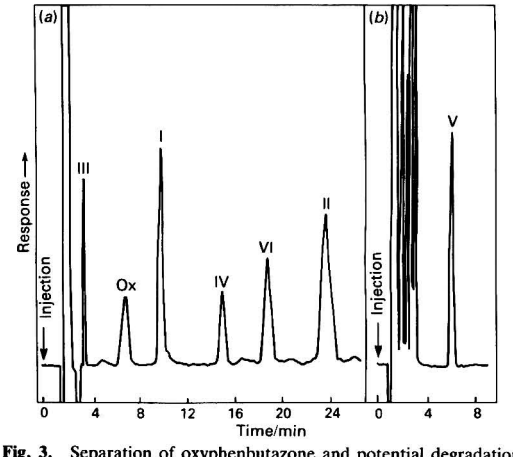


**Fig. 3.** Separation of oxyphenbutazone and potential degradation products under the optimised conditions. (*a*) Mobile phase A, 0.1 M THAM citrate buffer (pH 5.25) - acetonitrile - THF (65 + 29 + 6); flow-rate, 1.3 ml min$^{-1}$; pressure, 98 bar; detector sensitivity, 0.02 a.u.f.s.; chart recorder speed, 0.5 cm min$^{-1}$; wavelength, 239 nm; oxyphenbutazone, 1.15 μg ml$^{-1}$; **I**, 1.30 μg ml$^{-1}$; **II**, 1.75 μg ml$^{-1}$; **III**, 1.37 μg ml$^{-1}$; **IV**, 1.47 μg ml$^{-1}$ and **VI**, 1.30 μg ml$^{-1}$. (*b*) Mobile phase B, 0.1 M THAM citrate buffer (pH 5.25) - acetonitrile (45 + 55); flow-rate, 1.8 ml min$^{-1}$; pressure, 105 bar; detector sensitivity, 0.02 a.u.f.s.; chart recorder speed, 0.5 cm min$^{-1}$; wavelength, 239 nm; oxyphenbutazone 1.25 μg ml$^{-1}$; **I**, 1.42 μg ml$^{-1}$; **II**, 1.52 μg ml$^{-1}$; **III**, 1.17 μg ml$^{-1}$; **IV**, 1.22 μg ml$^{-1}$; **V**, 1.37 μg ml$^{-1}$; **VI**, 1.67 μg ml$^{-1}$

**Table 1.** Chromatographic parameters for oxyphenbutazone and its decomposition products

| Compound | Mobile phase A | | | Mobile phase B | | |
|---|---|---|---|---|---|---|
| | $k'$ | *H*/mm | *As* | $k'$ | *H*/mm | *As* |
| Ox.* | 2.63 | 0.460 | 1.16 | 0.35 | 0.067 | — |
| **I** | 4.11 | 0.077 | 1.00 | 0.43 | 0.062 | 1.00 |
| **II** | 11.32 | 0.040 | 1.16 | 1.08 | 0.042 | 1.20 |
| **III** | 0.74 | 0.166 | 1.00 | 0.00 | — | — |
| **IV** | 6.79 | 0.052 | 1.25 | 0.64 | 0.046 | 1.00 |
| **V** | | | | 3.26 | 0.043 | 1.00 |
| **VI** | 8.79 | 0.047 | 1.40 | 0.88 | 0.043 | 1.30 |

* Oxyphenbutazone

**Table 2.** Repeatability, sensitivity and detectability data for oxyphenbutazone and its decomposition products

| Compound | Repeatability, σ,% | Sensitivity/ mm μg$^{-1}$ | Detectability*/ μg |
|---|---|---|---|
| Ox.† .. .. | 0.72 | 2435 | 0.0040 |
| I .. .. .. | 0.75 | 6615 | 0.0015 |
| II .. .. .. | 1.26 | 3314 | 0.0030 |
| III .. .. .. | 0.68 | 11387 | 0.0010 |
| IV .. .. .. | 1.18 | 2041 | 0.0050 |
| V .. .. .. | 1.59 | 7299 | 0.0010 |
| VI .. .. .. | 0.89 | 3231 | 0.0030 |

* Determined in the presence of oxyphenbutazone (5 μg).
† Oxyphenbutazone.

**Table 3.** Recovery of oxyphenbutazone and its degradation products from pharmaceutical formulations

| Formulation | Compound | Initial content/mg | Amount added/mg | Amount found/mg |
|---|---|---|---|---|
| Ointment .. | ..Ox.* | 50.200 | — | — |
| | I | 0.034 | 0.131 | 0.161 |
| | II | — | 0.176 | 0.172 |
| | III | 0.009 | 0.138 | 0.142 |
| | IV | Traces | 0.148 | 0.003 |
| | V | — | 0.138 | 0.141 |
| | VI | — | 0.131 | 0.132 |
| Tablet .. | Ox.* | 94.000 | — | — |
| | I | 0.394 | 0.259 | 0.678 |
| | II | — | 0.385 | 0.375 |
| | III | 0.014 | 0.274 | 0.296 |
| | IV | Traces | 0.294 | 0.363 |
| | V | Traces | 0.326 | 0.325 |
| | VI | — | 0.259 | 0.268 |
| Capsule .. | Ox.* | 78.750 | — | — |
| | I | 0.065 | 0.195 | 0.264 |
| | II | — | 0.263 | 0.263 |
| | III | — | 0.206 | 0.212 |
| | IV | Traces | 0.221 | 0.216 |
| | V | — | 0.210 | 0.218 |
| | VI | 0.051 | 0.195 | 0.244 |

* Oxyphenbutazone

Methanol, acetonitrile and THF were of HPLC solvent grade. Distilled water was filtered through a 0.45-μm filter (Millipore).

### Standard Solutions

A mixed stock standard solution containing 250 μg ml$^{-1}$ each of oxyphenbutazone and I–VI was prepared in methanol. This solution was suitably diluted with methanol to give a concentration range from 1 to 10 μg ml$^{-1}$.

### Test Solutions

*Ointment*

An accurately weighed amount of about 250 mg of ointment was sonicated for 5 min with 25 ml of methanol in a 50-ml centrifuge tube. The emulsion was rotated at 4000 rev min$^{-1}$ for 10 min.

*Tablets*

The coatings of ten tablets were carefully removed with a cutter and the average mass of one core was determined. The ten cores were combined and powdered in a mortar. A core mass of about 20 mg was accurately weighed into a 50-ml centrifuge tube, then sonicated for 5 min with 25 ml of methanol. The suspension was rotated at 4000 rev min$^{-1}$ for 10 min.

*Capsules*

An accurately weighed amount of about 34 mg of capsule powder was sonicated for 5 min with 25 ml of methanol in a 50-ml centrifuge tube. The suspension was rotated at 4000 rev min$^{-1}$ for 10 min. After centrifugation, the supernatant from the ointment, tablets or capsules was injected on to the chromatograph without dilution for the determination of the degradation products, then diluted (1 + 399) in methanol for the determination of oxyphenbutazone.

### Chromatography

Duplicate injections (10 μl) of each standard solution and test solution were injected under the following isocratic conditions: mobile phase A, 0.1 M THAM citrate buffer (pH 5.25) - acetonitrile - THF (65 + 29 + 6); flow-rate 1.3 ml min$^{-1}$;

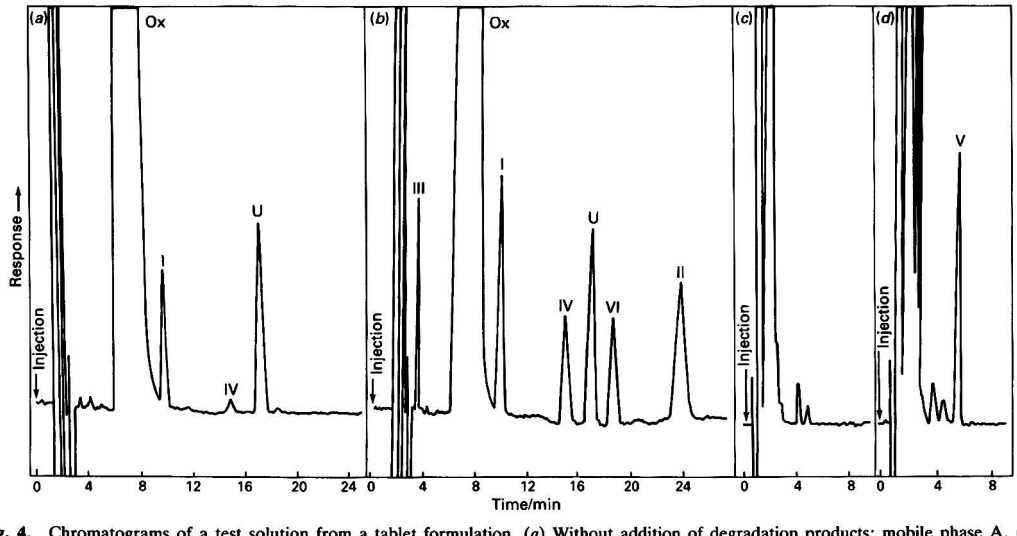


**Fig. 4.** Chromatograms of a test solution from a tablet formulation. (*a*) Without addition of degradation products; mobile phase A. (*b*) Spiked with **I–IV** and **VI**; mobile phase A. (*c*) Without addition of degradation products; mobile phase B. (*d*) Spiked with **I–VI**; mobile phase B. U, unidentified decomposition product

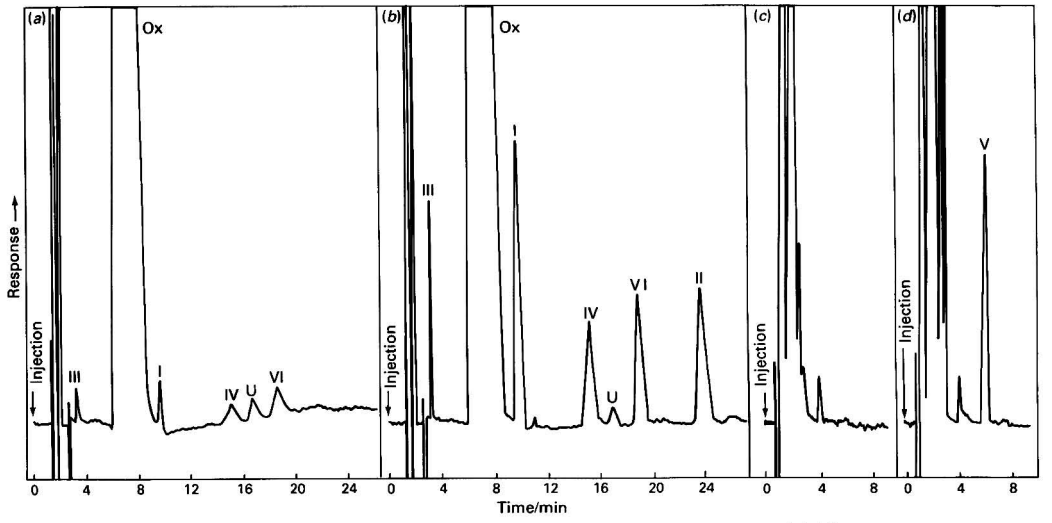


**Fig. 5.** Chromatograms of a test solution from capsule formulation. Chromatograms (*a*)–(*d*) as in Fig. 4

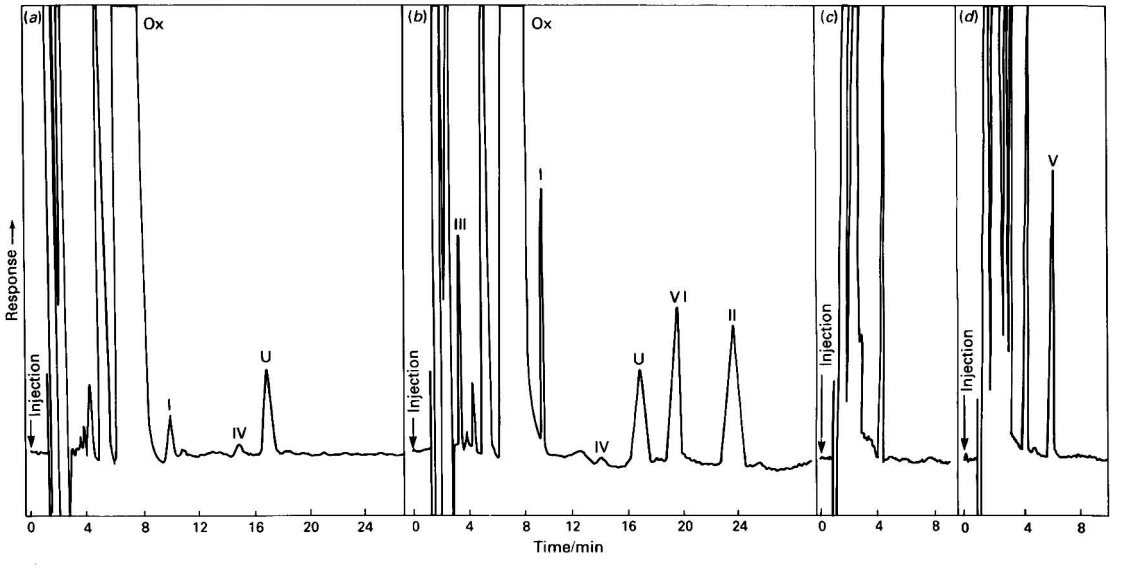


**Fig. 6.** Chromatograms of a test solution from an ointment. Chromatograms (*a*)–(*d*) as Fig. 4

pressure, 95 ± 0.1 bar; chart recorder speed, 0.5 cm $min^{-1}$; detector sensitivity, 0.04 or 0.02 a.u.f.s.; detection wavelength, 239 nm; mobile phase B, 0.1 M THAM citrate buffer (pH 5.25) - acetonitrile (45 + 55); conditions as above except flow-rate, 1.8 ml $min^{-1}$; pressure, 105 ± 0.1 bar.

## Results and Discussion

Specimen chromatograms of a standard solution recorded at 239 nm (a suitable wavelength for the simultaneous determination of all the compounds), using mobile phases A and B, are given in Fig. 3(*a*) and (*b*), respectively.

Table 1 gives the chromatographic data for a mixed standard solution of oxyphenbutazone and its decomposition products (10 μg $ml^{-1}$) expressed as the capacity factor, $k'$, the theoretical plate height, $H$, and the asymmetry factor, $As$, under the conditions used in this study. $As$, was calculated using the equation $As = b/a$, where $b$, is the distance after the peak maximum and $a$ the distance before the peak maximum, both being measured at 10% of the total peak height.

The stability of a solution of oxyphenbutazone and its decomposition products was tested by separately injecting on to the chromatograph, at different time intervals, a solution of oxyphenbutazone (500 μg $ml^{-1}$) and **I–VI** (10 μg $ml^{-1}$) in methanol. After 4 h at ambient temperature under diffused light, no decomposition was observed for oxyphenbutazone

and **I–VI** within the limit of sensitivity of the method (at 0.02 a.u.f.s.).

The stability of test solutions of the ointment, tablet core and capsule formulation (equivalent to 500 μg $ml^{-1}$) was also investigated. These solutions can be kept for 6 h without any detectable decomposition.

### Validation of the HPLC Procedure

The linearity of the response was examined by plotting the peak-height measurement for each solute against solute concentration in the range 0–100 μg $ml^{-1}$ for each compound. The calibration graph was rectilinear and passed through the origin in all instances. The correlation coefficient of the linear regression analysis was higher than 0.999 for each compound.

The repeatability, assessed by five replicate analyses of a mixed standard solution (10 μg $ml^{-1}$) and expressed as the coefficient of variation, is shown in Table 2. The sensitivity, defined as the change in the peak height (mm) measured at the maximum detector sensitivity resulting from a concentration change of one unit (μg), is also given in Table 2, together with the detectability, defined as the amount of compound that yields a signal to noise ratio of 2. The limit of determination can be evaluated as about three times the detectability.

Commercial formulations were spiked with known amounts of **I–VI** (0.25% of each with respect to the theoretical oxyphenbutazone content) and these spiked formulations were treated as indicated in the method. The chromatograms obtained are shown in Figs. 4–6 and average results of duplicate injections are given in Table 3. Satisfactory results were obtained except for **IV** in the ointment, probably because a physical and/or a chemical interaction took place. Attempts to solve this problem using different extraction solvents in the procedure (acetonitrile, the mobile phase solvent system, aqueous alkaline solutions) were unsuccessful. In addition, oxyphenbutazone was very unstable in these solvents.

Inert interference corresponding to a check on the placebo effect was carried out by treating a placebo of tablets, capsule formulation and the ointment as required in the method. No interference was observed.

## Conclusions

The proposed procedure allows the detection and determination of the potential degradation products of oxyphenbutazone at trace levels. The method is more sensitive than TLC,[1] as less than 0.1% of the decomposition products (with respect to oxyphenbutazone) can be quantified. In addition, the sample preparation is simple and rapid and allows the method to be used easily for routine control purposes.

## References

1. Fabre, H., Ramiaramanana, A., Blanchin, M. D., and Mandrou, B., *Analyst*, 1985, **110**, 1289.
2. Fabre, H., Mandrou, B., and Eddine, H., *J. Pharm. Sci.*, 1982, **71**, 120.

**Note**—Reference 1 is to Part I of this series.

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# Prevention of Artifactual Formation of Nitrosamines During the Analysis of Baby Bottle Rubber Nipples

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It has been found that considerable amounts of nitrosamines may be formed as artifacts during the analysis of rubber nipples by a method that involves Soxhlet extraction of the samples with dichloromethane. The extent of such formation was monitored by incorporating morpholine as a marker amine and studying the formation of nitrosomorpholine, which varied between 9 and 80 ng per analysis depending on the type of sample analysed and the brand of dichloromethane used. The problem could be minimised by pre-testing dichloromethane for its *N*-nitrosation potential and by incorporating propyl gallate, an *N*-nitrosation inhibitor, in the method.

**Keywords:** *Volatile nitrosamine determination; artifactual formation; gas - liquid chromatography - thermal energy analysis; rubber nipple analysis; propyl gallate*

During the past few years, considerable effort has been spent by various researchers in developing sensitive and specific methods for the determination of trace levels of *N*-nitrosamines (mainly volatile nitrosamines) in baby bottle rubber nipples and pacifiers.[1–4] The reason for this intense interest in this field stems from the fact that most of the nitrosamines detected in these products are potent carcinogens in laboratory animals[5] and that trace amounts of these chemicals can migrate to liquid infant foods or infant saliva, thus posing a potential health hazard to infants using these products. For a detailed background on the subject the reader is advised to consult earlier publications on this topic.[1,2,6–8]

Like all other trace analyses, the analysis of rubber nipples for volatile nitrosamines is a complex and difficult task. The analyst has to pay attention to the usual problems such as developing efficient extraction and clean-up techniques, avoiding contamination from reagents and glassware and developing sensitive and specific detection methods. The situation is further complicated by the fact that some of the nitrosamines for which the analysis is carried out may be formed as artifacts during work-up of the samples or during final analysis (*e.g.*, in the hot injector of the gas chromatograph) of the extract by gas - liquid chromatography (GLC). This can happen because the rubber products themselves may contain the necessary precursors (amines and nitrosating agents) for nitrosamine formation, or nitrogen oxide gases ($NO_x$) present in the air can enter into the system and nitrosate the amines in the nipple extracts. For this reason, additional precautions have to be taken and safeguards have to be incorporated in the methods to minimise or eliminate such formation. Krull *et al.*[9] discussed various ways of minimising artifactual formation during analysis, and emphasised the importance of correctly determining the concentration of carcinogenic nitrosamines in consumer products.

The first evidence that artifactual formation of nitrosamines might pose a problem during analysis of rubber nipples was observed by Sen *et al.*[3] while comparing two different methods of analysis. It was noted that one of the methods that incorporated a nitrosation inhibitor and employed a simpler extraction technique (avoiding Soxhlet extraction) gave much lower values for nitrosamines in some samples than those obtained by another method[2] that did not include any nitrosation inhibitor and employed Soxhlet extraction with dichloromethane. Although the latter method had been tested[2] for artifactual formation by adding (after Soxhlet extraction) nitrite and morpholine to nipple extracts and analysing for possible formation of *N*-nitrosomorpholine, it was felt that the test was inadequate because the nitrosamine precursors were not added at the start of the analysis. Therefore, the possibility of artifactual formation in the latter method could not be completely ruled out; the need for further work was suggested.

In this paper we present further evidence to indicate that artifactual formation of nitrosamines can indeed take place during the extraction of rubber nipples with dichloromethane. An improved method is suggested that greatly alleviates the problem.

## Experimental

### Apparatus

A Model 502 thermal energy analyser (TEA) (Thermo Electron Corp., Waltham, MA, USA) coupled to a Varian VISTA 6000 gas chromatograph was used for the determination of volatile nitrosamines. The gas chromatographic columns and operating conditions were similar to those reported previously.[7] The TEA was operated in the GLC mode with a stainless-steel cold trap immersed in liquid nitrogen and a furnace temperature of 475 °C. The vacuum chamber pressure was *ca.* 1.8 Torr.

### Reagents and Standards

Glass-distilled dichloromethane (DCM) was purchased from three suppliers. Of these, two were sold as nitrosamine-free reagents and for convenience these will be referred to as brands A and C. The third (brand B) was of the regular glass-distilled variety and contained cyclohexene as a preservative. It is understood that the DCM of brands A and C did not contain any known preservative, but they may have been processed by special techniques. As commercial DCM had previously been shown[10] to be contaminated with *N*-nitrosomorpholine, each bottle of DCM was redistilled from an all-glass apparatus and tested for nitrosamine contamination before use.

All other reagents used were of analytical-reagent grade. To ensure the absence of nitrosamine contamination, a reagent blank was used with each batch of new reagents and analysed by GLC - TEA. Highly activated basic alumina (ICN

Nutritional Biochemicals, Cleveland, OH, USA) was prepared by heating overnight at 500 °C, cooling in a desiccator and then storing in a stoppered flask. It was used without any addition of water for the purification of certain batches of DCM, as will be discussed later. Ascorbyl palmitate and propyl gallate were obtained from ICN, K & K Laboratories, Plainview, NY, USA, and Eastman Chemical Products, Kingsport, TN, USA, respectively. Morpholine was obtained from BDH Chemicals, Poole, UK.

Dilute standards (*ca.* 10 μg ml$^{-1}$ of each in ethanol) of a mixture of seven volatile nitrosamines and a separate standard of *N*-nitrosodipropylamine (100 μg ml$^{-1}$) were purchased from Thermo Electron Corp. The mixture consisted of the following: *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosomorpholine (NMOR). The solutions were appropriately diluted with DCM before use.

**Note**—Reference to a brand or company name does not constitute endorsement by the Consumer and Corporate Affairs of Canada or by Health and Welfare Canada over others of a similar nature not mentioned.

### Samples

All of the samples, except one, were purchased locally during October - December, 1984, or procured by the Inspector of the Department of the Consumer Corporate Affairs. These are identified by capital letters (D, H, F, etc.). Only a few samples of brand H from a recent (February, 1985) production lot were obtained directly from the manufacturer. Those identified by lower-case letters (a, e, etc.) were left-over samples from an AOAC collaborative study in which we participated during June - July, 1984.

**Caution**—As all of the nitrosamine standards mentioned above are potent carcinogens, extreme precaution should be taken when working with or handling the chemicals. Contact with skin or inhalation of vapour must be avoided. All solutions containing nitrosamines should be destroyed by a well established procedure[11] before disposal.

### Analysis of Rubber Nipples

*Method* 1

The method was essentially the same as that described by Havery and Fazio[2] but later modified to include the addition of 2 g of barium hydroxide to prevent foaming during distillation. Basically it consisted of (a) overnight soaking of the cut nipple pieces with DCM followed by Soxhlet extraction, (b) distillation of the DCM extract from an aqueous alkaline solution, (c) re-extraction of the aqueous distillate (containing the volatile nitrosamines) with DCM, (d) concentration of the DCM extract to a small volume (*ca.* 1 ml) using a Kuderna - Danish concentrator and a micro-Snyder column (for the final concentration step to 1 ml) and (e) GLC - TEA analysis of the final extract.

In this study, 1.0 ml of NDPA (10 ng ml$^{-1}$) was added as an internal standard to each sample at the beginning of the analysis. This was carried out only to check the performance of the method; the final results were not corrected for percentage recoveries of NDPA. Also, in some instances, 1 mg of morpholine (in 1 ml of DCM, brand B) was added at the start to the sample as a marker amine to monitor the extent of artifactual formation of nitrosamines.

*Method* 2

The method has been described in detail elsewhere.[3] In summary, it consisted in overnight extraction of the sample with DCM in the presence of 100 mg of ascorbyl palmitate as an *N*-nitrosation inhibitor, rinsing of the nipple pieces using a special extraction technique that did not involve Soxhlet extraction, concentration of the extract to 1 ml as in method 1 and final analysis by GLC - TEA.

### Testing the Effect of Different Brands of DCM on Nitrosamine Levels in Rubber Nipples as Determined by Method 1

As nitrosamine levels may vary from nipple to nipple, such tests were always carried out in pairs using half of a nipple or using aliquots from a composite mixture of cut nipple pieces. Also, everything else such as size of the distillation and Soxhlet flasks, size of the Soxhlet extractor and other reagents was kept constant. The size of the apparatus was kept as small as possible so as to avoid an excessive reduction in volume of DCM from the Soxhlet extraction flask (at the bottom) that, otherwise, could have caused overheating. The paired experiments were carried out simultaneously in the same fume hood. This ensured identical atmospheric conditions such as $NO_x$ levels that may have an influence on the formation of nitrosamines during Soxhlet extraction. A 1-ml volume each of NDPA internal standard and morpholine marker amine were also routinely added at the start to check the performance of the method and to monitor the artifactual formation of NMOR, respectively. Prior to this, the samples were analysed without any addition of morpholine to ensure the absence of pre-formed NMOR in them.

Morpholine blanks (1 mg) were also run in the same manner with different lots and brands of DCM, the only difference being that no nipples were present in these tests. The Soxhlet extracts in these instances were concentrated directly (omitting the aqueous distillation) and analysed by GLC - TEA.

### Testing the Effect of Propyl Gallate (PG)

While carrying out this test, all the factors including the DCM solvent were kept constant. Half of a nipple was analysed by the above method[2] and the other half by the same method but with added PG (100 mg). The stoppered flask containing the nipple pieces, PG and DCM was gently shaken (to aid in dissolving PG) overnight in the dark, while the other (without PG) was allowed to sit in the dark without shaking as specified in the original method.[2] The other steps were unchanged.

## Results and Discussion

The data (Table 1) indicate that the levels of nitrosamines detected in a sample of rubber nipple by method 1 can vary widely, depending on the type of DCM used for the analysis. The variation in results due to the use of different DCM could not all have been due to random errors because duplicate results (using brand B DCM) run under such controlled conditions usually were within 10%. Therefore, the difference (considered to be significant if $\geqslant \pm 30\%$) in the two sets of results in Table 1 was probably caused by artifactual formation, the extent of which varied from brand to brand of DCM and, to a smaller extent, from bottle to bottle of DCM within the same brand. This theory was also supported by the fact that there was a concomitant rise in the artifactual formation of NMOR from added morpholine for a particular DCM used for the analysis. As neither the morpholine nor the nipples (when analysed alone) contained any detectable amount of NMOR, the NMOR detected in these experiments must have formed as an artifact. Similar conclusions could also be drawn from the results for the morpholine blanks (Table 2). Some typical chromatograms obtained from these experiments are shown in Fig. 1.

Although the exact nature of the nitrosating agent(s) responsible for the artifactual formation of nitrosamines in the above method is not known, it is well established that DCM is an excellent medium for *N*-nitrosation.[12,13] Both inorganic

**Table 1.** Effect of different brands of DCM on the levels of nitrosamines in various nipples and that of artifactually formed NMOR in the presence of added morpholine

| Brand of nipple | Brand of DCM used for analysis | Nitrosamines detected in nipples/ μg kg$^{-1}$ | NMOR formed as an artifact*/ng | Recovery of NDPA internal standard, % |
|---|---|---|---|---|
| e | B | NDEA, 5.5 | 9.2 | —‡ |
| e | C | NDEA, 11.7 | 67.8 | — |
| b | B | Negative | — | 97.5 |
| b | A | NDMA, 55.3; NDEA, 4.3; NDBA, 4.8; NPYR, 6.7; NMOR, 3.5 | — | 100 |
| e | B | NDEA, 3.9; NMOR, 2.3 | — | 75 |
| e | A | NDEA, 10.0; NMOR, 2.2 | — | 80 |
| H | B | NDMA, 9 | 10.9 | 95.2 |
| H | A | NDMA, 83 | 74.3 | 96.3 |
| H | A, passed through basic alumina | NDMA, 38.9 | 41.8 | 103 |
| H | A, purified by sulphamic acid wash and alkali wash, then dried over anhydrous sodium sulphate | NDMA, 8.2 | 11.5 | 80.2 |
| H | B | NDMA, 8.3 | 11.8 | 83.3 |
| H | C | NDMA, 19 | 23.5 | 93.8 |
| H | C, passed through basic alumina | NDMA, 7.9 | 11.2 | 75.7 |
| Blanks | A, B, or C (without added morpholine) | All negative | | |

* When analysis was carried out with 1 mg of added morpholine as a marker amine.
† Not included.

**Table 2.** Artifactual formation of NMOR from added morpholine in blank runs by method 1

| NMOR formed from 1 mg of added morpholine/ng | | |
|---|---|---|
| DCM A | DCM B | DCM C |
| 30.6 | 16.9 | 13.7 |
| 47 | 22.9 | 15.7 |
| >60 | | 19.6 |
| 11.5 (after special processing)* | 41.8 | 80.3 |
| | 14.5 | 38.6 |
| | 14.5 | 31.3 |
| | 12.4 | 24.1 |
| | | 14.2 (after special processing)* |
| | | 59.6 |

* See text.

nitrite and $NO_x$ gases can efficiently nitrosate secondary amines in DCM.[12,13] The nitrosating agents could have originated in several ways. Firstly, they could have been present in the nipples[1,14] or formed as a result of thermal degradation during Soxhlet extraction.

Transnitrosation by organic nitro or nitroso compounds present in the nipples might have been partly responsible for the artifactual formation of nitrosamines observed in this study. This was demonstrated by analysing (by method 1) a 5-g aliquot of cut nipple pieces (brand H) with 1 mg of added *N*-nitrosodiphenylamine, a well known rubber curing ingredient,[14] and showing increased formation of NDMA during the analysis (10000 μg kg$^{-1}$ compared with 13 μg kg$^{-1}$ in the absence of added *N*-nitrosodiphenylamine). This suggested that there was enough amine precursors present in the sample that reacted with *N*-nitrosodiphenylamine, which is an excellent transnitrosating agent, to produce the excess of NDMA. From the excessively high result obtained in the above experiment, one would expect the formation of substantial amounts of NDMA even in the presence of much smaller

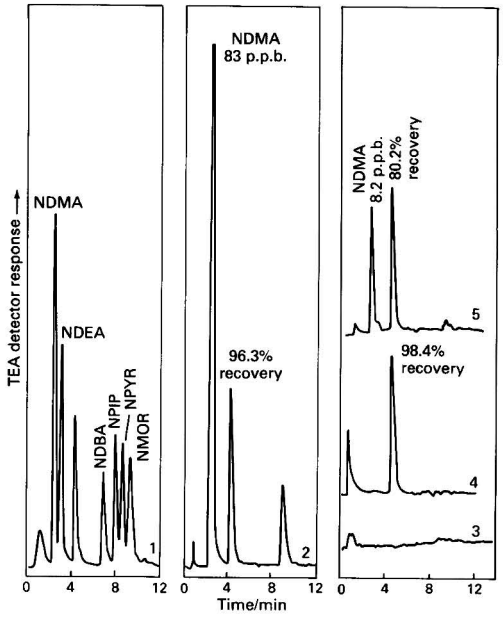


**Fig. 1.** Typical chromatograms showing the effect of special processing of DCM on the results obtained by method 1. 1, Nitrosamine standards; 2, a nipple (brand H) analysed using DCM of brand A (before special processing or clean-up); a total of 74.3 ng of NMOR was formed from the added morpholine marker amine; 3, direct injection of morpholine solution showing the absence of NMOR; 4, DCM of brand A (before special processing) reagent blank taken through all the steps of method 1 (without morpholine marker amine); and 5, above nipple analysed using specially processed (sulphamic acid and alkaline washes, etc.) DCM from same bottle as above; only 11.5 ng of NMOR were formed. Percentage recoveries refer to those with added NDPA internal standard. For details, see text and Table 1

amounts (*e.g.*, 10–100 μg) of *N*-nitrosodiphenylamine. Alternatively, $NO_x$ and $O_2$ in the air could have entered the system during Soxhlet extraction. According to Mirvish,[13] nitrosation of certain amides by $NO_x$ in DCM is about 30000 times faster than that by nitrous acid in aqueous solution. Therefore, even trace amounts of $NO_x$ gases in the air could form significant amounts of nitrosamines because the reaction is almost quantitative.

The fact that NMOR could form in the absence of rubber nipples (*e.g.*, morpholine blanks) led us to speculate that $NO_x$ gases in the air might be partly responsible for such artifactual formation. As the extent of such formation varied with different brands of DCM (run side by side), the existence of some other factors such as catalysts (*e.g.*, trace amounts of HCl) or of other nitrosating agents in the DCM was a possibility. Other possible mechanisms include (a) participation of certain transition metals (*e.g.*, zinc dithiocarbamates are used as additives in rubber manufacture) as catalysts of *N*-nitrosation[14,15] and (b) formation of amine - NO Drago complexes followed by oxidation by $O_2$ (in the air) to the nitrosamines.[16]

As reagent blank determinations (Fig. 1) carried out according to the protocol of method 1 cannot distinguish a good from a bad DCM (both give negative blanks), we have developed a procedure that allows one to pre-test the DCM. This is done by carrying out a morpholine blank determination as described under Experimental (Table 2). The greater the extent of NMOR formation in such a test, the greater will be the chance of artifactual formation of nitrosamines during analysis of rubber nipples using the particular DCM. The best commercially available glass-distilled DCM tested gave a value of *ca.* 10 ng of NMOR in such tests. Therefore, it is recommended that any DCM that gives a significantly higher value for NMOR formation (say >15 ng) should not be used for such analyses, otherwise the results in extreme instances (*e.g.*, sample H, Table 1) could be inflated by as much as 950%. To our knowledge, the possibility of this happening has not been investigated or reported previously.

It should be emphasised that neither of the DCMs of brand A or C was of sub-standard quality. Both were of glass-distilled varieties and both were redistilled and tested to be nitrosamine free before use. Also, both gave negative reagent blanks (Fig. 1D) when taken through all the steps of method 1. Therefore, without a knowledge of the data presented here, an analyst would have no justification for rejecting them for use in the analysis of rubber nipples for nitrosamines.

As all the DCMs were redistilled before use, the responsible nitrosating agent or catalyst in the DCM must be volatile, and should not be removed by distillation. Therefore, alternative methods were developed to purify DCM. A 1-l volume of brand C DCM (initially forming 25–30 ng of NMOR in the morpholine test) was purified by passage through a column containing 50 g of highly activated (activity I) basic alumina. The purified DCM on re-testing formed only 14 ng of NMOR. Similar treatment through alumina, however, was only partially effective for a sample of brand A DCM that initially formed >60 ng of NMOR in the morpholine test (Table 2). This DCM was further purified by shaking vigorously for 5 min in a separating funnel with 1% sulphamic acid (prepared in 0.5 M $H_2SO_4$), back-washing with 1 M KOH solution, and drying over anhydrous sodium sulphate. The treatment greatly improved the DCM, which on re-testing gave a very low value of 11.5 ng of NMOR in the morpholine test (Table 2).

These two specially purified DCMs were further tested for the analysis of rubber nipples and the results were compared with those obtained with unpurified DCM from the respective bottles (Table 1). The results (Table 1, nipple H, DCM of brands A and C) clearly indicate a noticeable reduction in the artifactual formation of NMOR, and also lower results for the nipples compared with those obtained with the corresponding unpurified DCM. The respective new (with purified DCM) results were also comparable to those obtained with the brand B DCM, the best commercially available. The fact that it was possible to purify two poor lots of DCM and obtain results comparable to those obtained with a third brand (B) of good DCM lends further support to the conclusion that the results obtained by method 1 are subject to extreme variations depending on the quality of DCM used for the analysis. Therefore, the importance of pre-testing DCM before starting the analysis cannot be overemphasised.

Next, the possibility of similar artifactual formation of nitrosamines that could be occurring even with the best DCM, *i.e.*, brand B, was investigated. In previous studies with cured meats,[17] fried bacon,[17] beer[18] and rubber nipples,[3] researchers have recommended incorporation of *N*-nitrosation inhibitors in the analytical protocols in order to minimise such formation. After some preliminary trials with various inhibitors (ascorbic acid, ascorbyl palmitate, α-tocopherol, pyrrole and propyl gallate), propyl gallate (PG) was selected for this purpose because it gave the most consistent results. The results of several analyses in which duplicate halves of rubber nipples were analysed with or without PG are presented in Table 3. As can be seen from the data, the inclusion of 100 mg of PG in method 1 gave much lower values for nitrosamines in most instances, suggesting artifactual formation of nitrosamines in its absence. In a few instances, this was also confirmed by adding morpholine as a marker amine (Fig. 2). PG also reduced the formation of NMOR. It should be emphasised that this phenomenon was not observed with all nipples tested (*e.g.*, samples D, F, $H_6$ and i). This was particularly noticeable with the most recent nipple of brand H ($H_6$). Probably the nitrosamines in these instances were already present in the nipples or the necessary precursors were absent (as a result of the introduction of improved rubber curing practices).

The extent of inhibition of nitrosamine formation in the presence of PG varied depending on the brand of DCM used and also with the type of nipple. Even with the best commercially available DCM (brand B) one could obtain a result that was 375% higher (Table 3, sample $H_4$) if PG was omitted. In an extreme instance (*e.g.*, sample $H_5$ with brand A DCM) the difference was 700%. The addition of PG did not in any way affect the performance of the method, nor did it affect the recoveries of added nitrosamines. In the presence of PG, the recoveries of all seven volatile nitrosamines (see Reagents and Standards) added to rubber nipples at *ca.* 20 μg $kg^{-1}$ levels and also that of NDPA internal standard added to all samples were excellent (80–100%). This ruled out the possibility of any loss of nitrosamines due to breakdown or interaction with PG.

A few analyses were carried out (using brand H nipple) in which PG or *N*-nitrosodiphenylamine was added at various stages of method 1. The results suggest that, in the absence of PG, artifactual formation of nitrosamines can take place during both DCM extraction and alkaline distillation. As information regarding the detailed composition of various nipples was not easily available, it was difficult to investigate the problem more thoroughly. Further work might be desirable to understand fully the mechanism of artifactual formation observed in this study.

In a few limited instances the results obtained by the improved method 1 (with PG and pre-testing DCM) were compared with those obtained by method 2 (Table 4). They were in excellent agreement. In a previous study[3] with rubber nipples it had been observed that method 1 (without PG) could give results higher than or comparable to (never lower than) those obtained by method 2, which included an *N*-nitrosation inhibitor. The findings presented in this paper have been helpful in explaining the reasons behind these discrepancies. The occasional higher results previously observed with method 1 were probably due to artifactual formation. This was also substantiated by the data in Fig. 3,

**Table 3.** Inhibition of artifactual formation of nitrosamines by PG during analysis of rubber nipples for volatile nitrosamines

| Brand of nipple* | DCM brand used for analysis | Volatile nitrosamines detected: Nitrosamine | Amount by method 1/ μg kg⁻¹† | Amount by method 1 with PG/μg kg⁻¹† |
|---|---|---|---|---|
| $H_1$ | C | NDMA | 60 (81.3%) | 13.6 (93.6%) |
| $H_2$ | C | NDMA | 78.1 (90.3%) | 15.4 (96.9%) |
| $H_3$ | B | NDMA | 9.8 (86.5%) | 4.0 (98.0%) |
| $H_4$ | B | NDMA | 13.5 (76.7%) | 3.6 (76.7%) |
| $H_5$ | A | NDMA | 107 (85%) | 14.0 (93.8%) |
| $H_6$‡ | B | NDMA | 2.0 (90%) | 2.0 (85%) |
| | | NDEA | 1.5 | 1.5 |
| | | NMOR | 4.0 | 4.0 |
| a | C | NDMA | 79.1 (85.4%) | 10.3 (94.4%) |
| a | B | NDMA | 14.5 (92.2%) | 5.9 (87.5%) |
| a | B | NDMA | 8.5 (100%) | 3.6 (100%) |
| F | B | NDBA | 181 (102.7%) | 167 (89.3%) |
| | | NPIP | 39.4 | 36.3 |
| g | B | — | Negative (86.7%) | Negative (92%) |
| i | B | NDBA | 81.9 | 99.4 |
| e | B | NDEA | 15.0 (81%) | 5.6 (77%) |
| d | B | NDEA | 3.2 (75.6%) | 2.6 (85%) |
| D | B | NDBA | 87.7 (94%) | 79.3 (94.5%) |
| | | NPYR | 13.7 | 12.3 |
| | | NMOR | 10.9 | 12.3 |

* Different subscripts indicate samples from different lots or different nipples from the same lot.
† Figures in parentheses represent recoveries of NDPA internal standard.
‡ Obtained directly from the manufacturer in February 1985.

**Table 4.** Comparison of results obtained by improved* method 1 with those obtained by method 2

| Brand of nipple | NDMA detected/μg kg⁻¹†: Method 1 | Improved method 1 | Method 2 |
|---|---|---|---|
| H | 18.9 (89.2%) | 5.1 (82.5%) | 5.9 (97.5%) |
| a | 8.5 (100%) | 3.6 (100%) | 3.3 (97%) |
| a | 14.5 (92.2%) | 5.9 (87.5%) | |
| H‡ | 71.4 (82.1%) | — | 9.7 (98.5%) |

* Using brand B DCM and including 100 mg of PG.
† Figures in parentheses represent percentage recoveries of NDPA internal standard.
‡ Using brand C DCM.

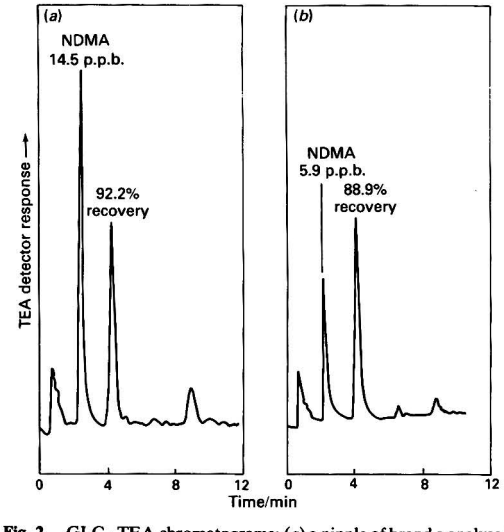


**Fig. 2.** GLC - TEA chromatograms: (*a*) a nipple of brand a analysed by method 1 using DCM of brand B; (*b*) the same nipple analysed in the presence of PG. About 24.3 and 12.6 ng of NMOR were formed, respectively, from the morpholine added in each instance

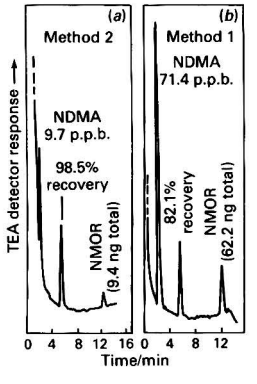


**Fig. 3.** GLC - TEA chromatograms of a nipple (brand H, see Table 4) analysed by (*a*) method 2 and (*b*) method 1 (with morpholine marker amine added in each instance) using DCM of brand C. Both the NDMA levels detected in the nipple and the amounts of NMOR formed were higher with method 1. The GLC column and conditions were slightly different from those in Figs. 1 and 2, hence the slight difference in retention times

which show increased formation of NMOR in method 1 compared with method 2.

It has been previously shown[3,18] that the use of a micro-Snyder column (instead of blowing down in a stream of nitrogen) for the final concentration from 4 to 1 ml and the use of a Graham condenser, which is more efficient than a straight-jacket condenser, during the distillation step can give consistently good recoveries of the volatile nitrosamines. It is therefore recommended that both of these modifications also be incorporated in the improved version of method 1.

Finally, it should be emphasised that this work should not be viewed as an undue criticism of method 1. The main objective was to determine the cause of variations in results produced by method 1 and to improve it. Basically, it is a good method that needed refinement. With the modifications suggested above and those mentioned earlier (*i.e.*, pre-testing DCM and including PG), the method would improve greatly,

and should give more precise and accurate results. Also, the effect of PG on nitrosamine levels detected by method 1 should be tested for any new product not analysed before. This will offer a safeguard against any possible catalytic influence it might have on nitrosamine formation in the presence of new rubber curing agents.

## References


1. Spiegelhalder, B., and Preussmann, R., *IARC Sci. Publ.*, 1982, No. 41, 231.
2. Havery, D. C., and Fazio, T., *Food Chem. Toxicol.*, 1982, **20**, 939.
3. Sen, N. P., Seaman, S. W., Clarkson, S. G., Garrod, F., and Lalonde, P., *IARC Sci. Publ.*, 1985, No. 57, 51.
4. Billedeau, S. M., Thompson, H. C., Jr., Miller, B. J., and Wind, M. L., 98th Annual AOAC Meeting, October 29–November 2, 1984, Washington, DC, Abstract No. 252.
5. Preussmann, R., and Stewart, B. W., *in* Searle, C. E., *Editor*, "Chemical Carcinogens," Second Edition, ACS Monograph No. 182, American Chemical Society, Washington, DC, 1984, p. 643.
6. Babish, J. G., Hotchkiss, J. H., Wachs, T., Vecchio, A. J., Guttenmann, W. H., and Lisk, D. J., *J. Toxicol. Environ. Health*, 1983, **11**, 167.
7. Sen, N. P., Kushwaha, S. C., Seaman, S. W., and Clarkson, S. G., *J. Agric. Food Chem.*, 1985, **33**, 428.
8. Österdahl, B.-G., *Food Chem. Toxicol.*, 1983, **21**, 755.
9. Krull, I. S., Fan, T. Y., and Fine, D. H., *Anal. Chem.*, 1978, **50**, 698.
10. Eisenbrand, G., Spiegelhalder, B., Janzowski, C., Kann, J., and Preussmann, R., *IARC Sci. Publ.*, 1978, No. 19, 311.
11. Castegnaro, M., Eisenbrand, G., Ellen, G., Keefer, L., Klein, D., Sansone, E. B., Spincer, D., Telling, G., and Webb, K. S., *in* "Laboratory Decontamination and Destruction of Nitrosamines in Laboratory Wastes," *IARC Sci. Publ.*, 1982, No. 43.
12. Angeles, R. M., Keefer, L. K., Roller, P. P., and Uhm, S. J., *IARC Sci. Publ.*, 1978, No. 19, 109.
13. Mirvish, S., *in* Magee, P, N., *Editor*, "Nitrosamines and Human Cancer," Banbury Report No. 12, Cold Spring Harbor Laboratory, New York, 1982, p. 227.
14. International Agency for Research on Cancer, "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: The Rubber Industry," IARC, Lyon, 1982, Chapter IV, p. 89.
15. Croisy, A. F., Fanning, J. C., Keefer, L. K., Slavin, B. W., and Uhm, S.-J., *IARC Sci. Publ.*, 1980, No. 31, 83.
16. Hansen, T. J., Croisy, A. F., and Keefer, L. K., *IARC Sci. Publ.*, 1982, No. 41, 21.
17. Hotchkiss, J. H., Libbey, L. M., Barbour, J. F., and Scanlan, R. A., *IARC Sci. Publ.*, 1980, No. 31, 361.
18. Sen, N. P., Seaman, S., and Bickis, M., *J. Assoc. Off. Anal. Chem.*, 1982, **65**, 720.


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# Gas Chromatographic Determination of Triclopyr in Environmental Waters

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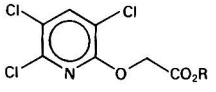
The reaction of $BF_3$-trifluoroethanol with an extract of triclopyr from an acidified sample solution to form the trifluoroethyl ester has been applied to the determination of triclopyr in environmental waters. The product is cleaned up by silica-gel column chromatography and determined by gas chromatography with electron-capture detection.

The detection and determination limits were 0.005 ng and 0.00025 μg ml$^{-1}$, respectively. The recovery and coefficient of variation were found to be 90–93% and less than 4%, respectively ($n$ = 7), for recovery experiments on river waters.

**Keywords:** *Triclopyr determination; gas chromatography; herbicide residue; river water; halogenated alkylation*

Triclopyr (3,5,6-trichloro-2-pyridyloxyacetic acid) is a hormone-type herbicide that is effective for the destruction of




arrowroots (*Pueraria thunbergiana*[1]) and deciduous shrubs. It is on the market as the triethylammonium salt (triclopyr-TEA) or butoxyethyl ester (triclopyr-BE). This herbicide, singly or mixed with Frenock (sodium 2,2,3,3-tetrafluoropropionate), is used extensively in woods and forests.[2] It is therefore necessary to determine the pollution of natural waters by this herbicide, for which purpose a simple and highly precise microanalytical method is required.

A microanalytical method for the determination of triclopyr has not been reported, although there are many reports of the determination of phenoxy herbicides,[3–16] which have similar properties to triclopyr. Phenoxy herbicides have a high polarity and low volatility, preventing the use of a direct GC method for their determination. Thus, they are subjected to GC or GC - MS after conversion into more volatile compounds, *i.e.*, alkyl esters,[3–9] halogenated alkyl esters[10,11] or halogenated aromatic esters.[12–16] In the environment triclopyr-BE is hydrolysed gradually into triclopyr-BE is more easily total amount of triclopyr and triclopyr-BE is more easily determined than are the two compounds separately. A method of determination has been developed in which triclopyr and triclopyr-BE in acidic, aqueous solution are extracted with diethyl ether, converted into the trifluoroethyl (TFE) ester, cleaned up by silica-gel column chromatography and determined by gas chromatography with electron-capture detection. (ECD - GC).

This method has sufficient sensitivity, precision and manageability to be applicable to environmental waters.

## Experimental

### Reagents

3,5,6-Trichloro-2-pyridyloxyacetic acid (triclopyr), triethylammonium 3,5,6-trichloro-2-pyridyloxyacetate (triclopyr-TEA) and butoxyethyl 3,5,6-trichloro-2-pyridyloxyacetate (triclopyr-BE) were obtained from Dow Chemical Japan. Standard solutions of each of these compounds were prepared from a concentrated solution of 1000 μg ml$^{-1}$ in acetone by diluting with acetone to 10, 1 or 0.1 μg ml$^{-1}$. Polychlorinated biphenyls (PCB), dibutyl phthalate, 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-methyl-4-chlorophenoxyacetic acid (MCP) were obtained from Wako Pure Chemical Co., Japan. Silica gel, Wako-gel S-1 from Wako Pure Chemical Co., Japan, was activated by heating for 12 h at 130 °C before use. A solution of 15% *m/V* boron trifluoride in 2,2,2-trifluoroethanol ($BF_3$-TFE) was obtained from Tokyo Kasei Co., Japan. Hexane, benzene, acetone and diethyl ether were of the grade suitable for detection of pesticide residues. All the other reagents were of guaranteed grade.

### Apparatus

The gas chromatograph was a Shimadzu Model GC-3BE equipped with an electron-capture detector ($^{63}$Ni), the Reacti-Therm was from Pierce Chemical Co., USA, the gas chromatograph - mass spectrometer was a Model JMS-D300 from Japan Electron Optics Laboratory Co., Japan, and the chromatographic column was 10 mm in diameter and 300 mm in length.

### GC Conditions

The stationary phase was 5% XE-60 on Chromosorb W (60–80 mesh), packed into a glass column (3 mm in diameter and 200 cm in length). The carrier gas was nitrogen with a flow-rate of 28 ml min$^{-1}$. The temperature was 155 °C for both the column and the detector and 200 °C for the injection port.

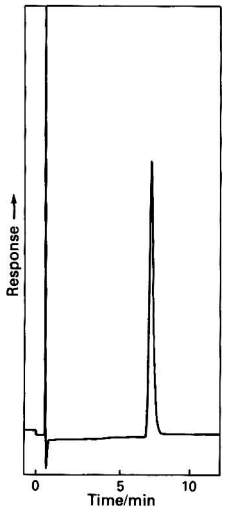



**Fig. 1.** Gas chromatogram of triclopyr-TFE. Column, 2 m, 5% XE-60; column and detector temperature, 155 °C; injection-port temperature, 200 °C; and carrier gas, $N_2$ at a flow-rate of 28 ml $min^{-1}$

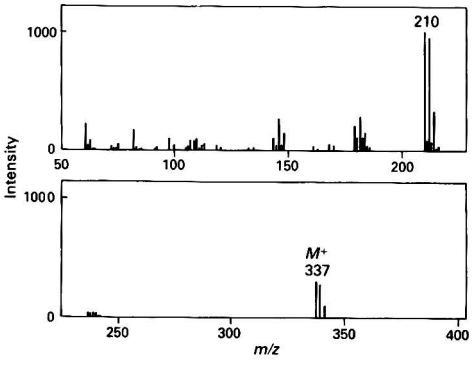



**Fig. 2.** EI mass spectrum of triclopyr-TFE. Column, 2 m, 5% XE-60; column temperature, 160 °C; injection-port and enricher temperatures, 200 °C; ion-source temperature, 250 °C; ionisation voltage, 70 eV; and carrier gas, He at a flow-rate of 40 ml $min^{-1}$

**Table 1.** Effects of reaction temperature and reaction time on the esterification of triclopyr

| Reaction time/ min | Esterification at reaction temperature, % | | | | |
|---|---|---|---|---|---|
| | 50 °C | 60 °C | 70 °C | 80 °C | 90 °C |
| 10 | 44 | 63 | 90 | 100 | 100 |
| 20 | 70 | 82 | 100 | 100 | 100 |
| 40 | 94 | 100 | 100 | 100 | 100 |
| 60 | 100 | 100 | 100 | 100 | 100 |
| 80 | 100 | 100 | 100 | 100 | 100 |

## Standard Procedure

The standard procedure consists of four steps: extraction, esterification, clean-up and determination.

**Table 2.** Effects of reaction temperature and reaction time on the ester-group exchange reaction

| Reaction time/ min | Exchange at reaction temperature, % | | | | |
|---|---|---|---|---|---|
| | 50 °C | 60 °C | 70 °C | 80 °C | 90 °C |
| 10 | 22 | 46 | 61 | 76 | 93 |
| 20 | 51 | 68 | 87 | 95 | 99 |
| 40 | 73 | 94 | 99 | 100 | 100 |
| 60 | 85 | 100 | 100 | 100 | 100 |
| 80 | 91 | 100 | 100 | 100 | 100 |

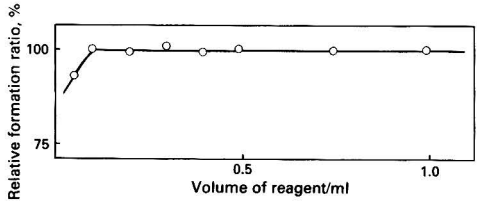



**Fig. 3.** Effect of amount of reagent on the esterification

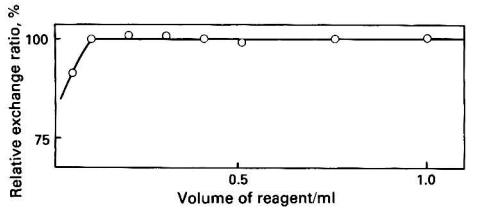



**Fig. 4.** Effect of amount of reagent on the exchange reaction of ester groups

*Extraction*

A 200-ml portion of the sample water is placed in a 300-ml separating funnel, to which 6 g of NaCl and 1 ml of 9 M $H_2SO_4$ are added and shaken to 10 min with each of two 50-ml portions of diethyl ether. The combined diethyl ether extracts are washed with 20 ml of 10% *m/V* NaCl solution, dried with anhydrous $Na_2SO_4$ and concentrated to less than 5 ml in a Kuderna - Danish (KD) concentrator.

*Esterification*

The concentrate is transferred into a 5-ml vial and the solvent is removed with a gentle stream of $N_2$. A 0.25-ml portion of $BF_3$-TFE is added to this vial, which is covered by a Teflon cap and placed on a Reacti-Therm at 80 °C and allowed to react for 1 h. After cooling, the contents are transferred into a 100-ml separating funnel with 30 ml of hexane, washed twice with 20 ml of 10% *m/V* NaCl solution, dried with anhydrous $Na_2SO_4$ and concentrated to less than 5 ml in the KD concentrator.

*Clean-up*

The concentrate is transferred on to a 3-g silica-gel column (10 mm i.d.) that has been slurry-packed in hexane. The column is washed with 100 ml of benzene - hexane (10 + 90), and the adsorbed compound is eluted with 100 ml of benzene - hexane (35 + 65).

*Determination*

The eluate is concentrated in the KD concentrator to less than 5 ml, diluted to the appropriate volume and subjected to ECD - GC.

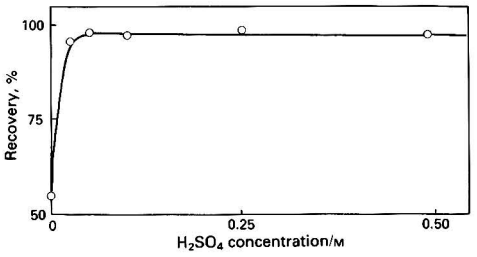



**Fig. 5.** Effect of acid concentration on the recovery

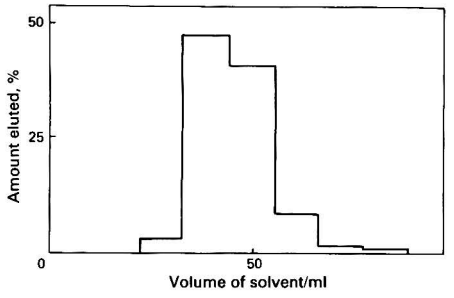



**Fig. 6.** Elution pattern of triclopyr-TFE

A blank test is conducted by the same procedure with 200 ml of distilled water.

## Results and Discussion

### Formation and Identification of Triclopyr-TFE

The following experiment was conducted in order to prevent tailing during gas chromatography and to produce ECD - GC analysis of high sensitivity.

A 1-mg mass of triclopyr and 0.5 ml of $BF_3$-TFE were placed in a vial, heated at 80 °C for 1 h, and extracted with hexane. To select the column conditions for the GC of the reaction product, a test was conducted in the range 150–200 °C on 2% OV-17, 2% OV-101, 5% SE-52, 5% DEGS and 5% XE-60. Fig. 1 shows the chromatogram obtained with XE-60, which was the best with respect to the peak shape and separation from coexisting substances. A mass spectral measurement gave a peak at $m/z$ = 337 corresponding to the molecular ion ($M^+$) of triclopyr-TFE (Fig. 2).

### Investigation of the Conditions for TFE Esterification

*Esterification*

To ascertain the optimum reaction temperature and time for the esterification, 2.5 ml (containing 2.5 μg) of triclopyr standard solution were placed in a 5-ml vial and the solvent was removed in a gentle stream of $N_2$. A 0.25-ml portion of $BF_3$-TFE was added to this vial, which was covered by a Teflon cap. Esterification was conducted at 50–90 °C for 10–80 min. As Table 1 shows, the ester was obtained quantitatively under the following reaction conditions: 60 min at 50 °C, 40 min at 60 °C, 20 min at 70 °C and 10 min at 80 or 90 °C.

The amount of triclopyr, the temperature and the reaction time were kept constant at 20 μg, 60 °C and 60 min, respectively, and the amount of the reagent was changed in the range 0.05–1 ml. Fig. 3 shows that the esterification is constant with a minimum amount of 0.1 ml of $BF_3$-TFE, but

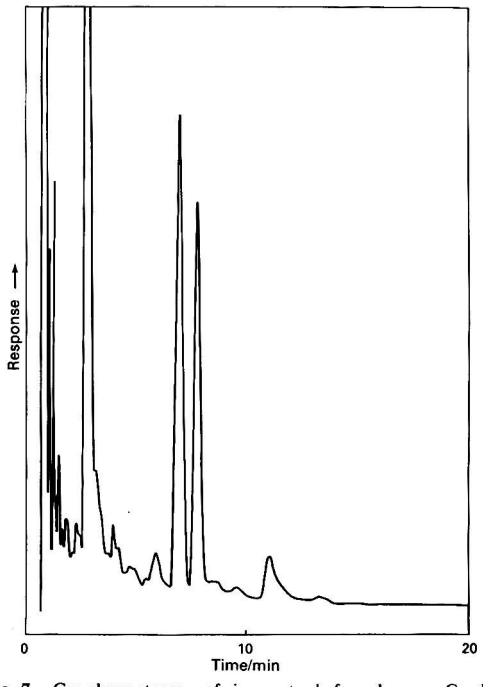



**Fig. 7.** Gas chromatogram of river water before clean-up. Conditions as in Fig. 1

0.25 ml of $BF_3$-TFE was used for actual samples in the event that they contain other substances that consume $BF_3$-TFE.

*Ester-group exchange reaction*

The effects of reaction temperature, reaction time and exchange ratio on the ester-group exchange reaction were investigated with 2.5 ml (containing 2.5 μg) of triclopyr-BE standard solution in a vial, under the same conditions as the esterification. Table 2 shows that the ester-group exchange reaction does not occur as easily as the esterification; even an 80-min reaction time at 50 °C did not yield a 100% exchange. Quantitative exchange requires 60 min at 60 °C, *ca.* 40 min at 70 °C, 40 min at 80 °C and 20 min at 90 °C.

The amount of reagent required for a quantitative reaction was found by using 20 μg of triclopyr-BE under the same conditions as the esterification. Although 0.1 ml of reagent was sufficient, 0.25 ml was used for the same reason as in the esterification (Fig. 4).

Hence the optimum conditions for esterification are as follows: addition of 0.25 ml of $BF_3$-TFE and reaction for 1 h at 80 °C.

### Investigation of the Extraction Conditions

*Extraction of triclopyr*

Diethyl ether was selected as the extraction solvent because it is easily removed after extraction.

The optimum acid concentration for extraction was determined in the following manner. A 2.5-ml volume of triclopyr-TEA standard solution (containing 2.5 μg) and different amounts of 9 M $H_2SO_4$ were added to 100 ml of distilled water to produce the final solution with $H_2SO_4$ concentrations of 0–0.5 M. Each was extracted with 50 ml of diethyl ether and then submitted to the standard analysis procedure. The relationship between the acid concentration and the recovery

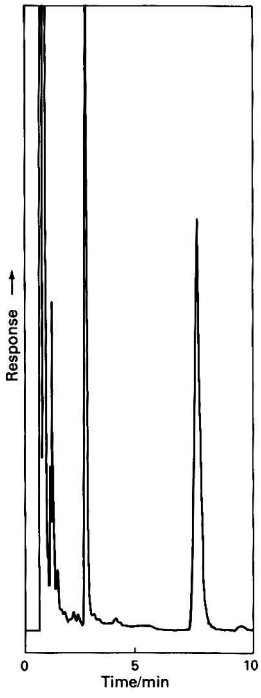



**Fig. 8.** Gas chromatogram of river water after clean-up. Conditions as in Fig. 1

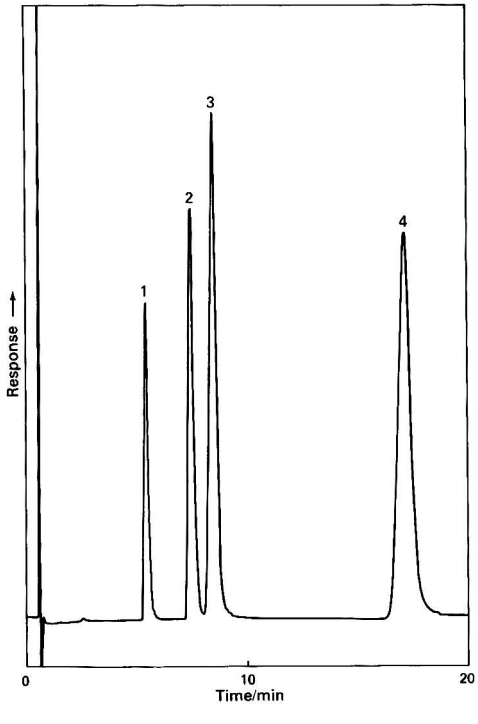



**Fig. 9.** Gas chromatogram of TFE esters of acid herbicides. Conditions as in Fig. 1. Peaks: 1, MCP, 2 ng; 2, triclopyr, 0.2 ng; 3, 2,4-D, 0.4 ng; 4, 2,4,5-T, 0.3 ng

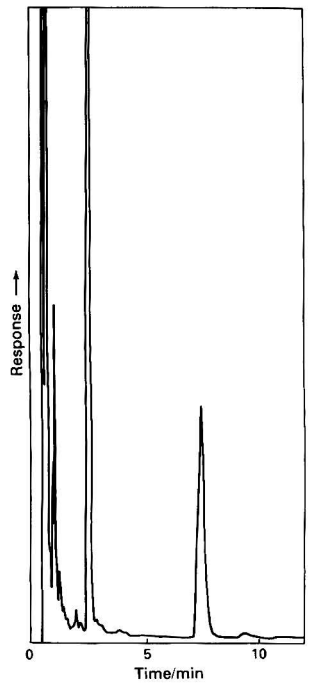



**Fig. 10.** Gas chromatogram of a river water. Conditions as in Fig. 1

was found and the results are shown in Fig. 5. The recovery of triclopyr-TEA was *ca.* 55% with no acid added and *ca.* 100% for an acid concentration above 0.025 M.

To determine the optimum salt concentration for extraction, the relationship between the salt concentration and the extraction ratio was investigated by changing the NaCl concentration in the range 0–30% *m/V*, keeping the acid concentration at 0.05 M. The recovery did not change. As environmental samples sometimes contain suspended substances that form emulsions which prolong the time required for separation, the addition of up to 3% NaCl solution was adopted to prevent such an emulsion formation.

*Extraction of triclopyr-BE*

Triclopyr-BE can be extracted by ordinary hydrophobic solvents and hence was extracted with diethyl ether, as was triclopyr-TEA. The dependence on the recovery with acid and salt concentrations was similarly investigated and was found to be independent of both acid and salt concentration over the range examined.

Hence 6 g of NaCl and 1 ml of 9 M $H_2SO_4$ were added to 200 ml of sample before extraction.

**Clean-up by Silica-gel Column Chromatography**

A variety of substances in natural waters can be simultaneously extracted and esterified by TFE and therefore can cause interference in the determination of triclopyr-TFE. Hence the esterification products were purified by silica-gel column chromatography.

Triclopyr-TFE (10 μg) was loaded on to a column (10 mm i.d.) containing 3 g of silica gel and eluted with 100 ml of benzene - hexane (10 + 90); no ester species were found in the eluate. The ester was completely eluted with a mixture (100 ml) of benzene - hexane (35 + 65) (Fig. 6).

**Table 3.** Results of the addition and recovery experiments for triclopyr-TEA and triclopyr-BE

| Sample | Compound | Amount added/μg | Recovery, % | Coefficient of variation ($n$ = 7), % |
|---|---|---|---|---|
| Distilled water | Triclopyr-TEA | 0.5 | 92 | 3.1 |
| | Triclopyr-BE | 0.5 | 93 | 2.7 |
| River water | Triclopyr-TEA | 0.5 | 90 | 3.5 |
| | Triclopyr-BE | 0.5 | 92 | 3.4 |

The clean-up procedure was as follows: the column was washed with 100 ml of benzene - hexane (10 + 90) and the ester was eluted with 100 ml of benzene - hexane (35 + 65). The usefulness of this procedure was demonstrated with 0.5 ml (containing 0.5 μg) of triclopyr standard solution in 200 ml of an actual river water (Figs. 7 and 8).

### Effect of Interfering Substances

The influence of phthalate esters, PCB, HCB, MCP, 2,4-D and 2,4,5-T, which can be extracted with diethyl ether, was examined by taking 5 μg of each substance in distilled water. The phthalate esters were extracted and esterified with TFE but eluted slightly from the silica-gel column. Their GC retention time (*ca.* 2.5 min) was considerably different from that of triclopyr-TFE. PCB and HCB were extracted but eluted by the washing solvent from the silica-gel column. MCP, 2,4-D and 2,4,5-T were extracted, esterified with TFE and eluted from the silica-gel column. However, they caused no interference as they have different GC retention times (Fig. 9).

### Calibration Graphs and Recovery Experiments

A calibration graph was prepared as follows: standard solutions containing 0.2, 0.4, 0.6, 0.8 and 1.0 μg of triclopyr were placed in 5-ml vials, the solvent was removed in a gentle stream of $N_2$, the residue was esterified with TFE and the amount of the ester measured by ECD - GC. The calibration graph was linear over the range examined. The detection limit was 0.005 ng with a 5-μl sample injection and the determination limit was 0.00025 μg ml$^{-1}$ using 200 ml of sample solution.

The recovery was examined by the standard procedure after adding a given amount of triclopyr to 200 ml of distilled water or river water without triclopyr. Table 3 shows that the proposed method is satisfactory, with recoveries of more than 90% and a coefficient of variation of less than 4%.

### Application to Real Samples

The effectiveness of the proposed procedure was tested by analysing actual samples of river water ($n$ = 12). It was found that 0.25 ml of $BF_3$-TFE was sufficient, its use in excess causing no interference, and that the coexistence of pollutants or natural organics did not interfere with the determination. A replicate experiment on a river water containing 0.001 μg ml$^{-1}$ of triclopyr gave a standard deviation of less than 4% ($n$ = 5). Fig. 10 shows the detection of 0.0015 μg ml$^{-1}$ of triclopyr in a river water sampled near a site where triclopyr had been released 1 week before.

## Conclusion

Triclopyr can be successfully extracted from acidified aqueous samples with diethyl ether and esterified with $BF_3$-TFE. Interfering substances can be eliminated by a clean-up method with a silica-gel column and using benzene - hexane as an eluting solvent. The recovery from actual river waters is 90–93%, with coefficients of variation of less than 4%. The detection and determination limits are 0.005 ng and 0.00025 μg ml$^{-1}$, respectively. The proposed procedure is also useful for the simultaneous determination of MCP, 2,4-D and 2,4,5-T if the clean-up method is modified.

The authors thank Dow Chemical Japan for the gifts of triclopyr, triclopyr-BE and triclopyr-TEA and Professor I. Matsuzaki and Messrs. S. Shimizu and H. Ozawa for many helpful suggestions.

## References

1. Makino, T., "Makino's Illustrated Flora in Color," Hokuyokan, Tokyo, 1982, p. 263.
2. Noyaku Yoran Henshu Iinkai, "Noyaku Yoran," Nippon Shokubutsu Boeki Kyokai, Tokyo, 1983, p. 159.
3. Howard, S. F., and Yip, G., *J. Assoc. Off. Anal. Chem.*, 1971, **51**, 970.
4. Khan, S. U., *J. Assoc. Off. Anal. Chem.*, 1975, **58**, 1027.
5. Thio, A. P., Kornet, M. J., Tan, H. S. I., and Tompkins, D. H., *Anal. Lett.*, 1979, **12**, 1009.
6. Cotterill, E. G., *Analyst*, 1982, **107**, 76.
7. Goetz, R., *Fresenius Z. Anal. Chem.*, 1983, **314**, 131.
8. Sekita, H., Takeda, M., Saito, Y., and Uchiyama, M., *Eisei Kagaku*, 1982, **28**, 219.
9. Chmil, V. D., *Zh. Anal. Khim.*, 1981, **36**, 1121.
10. Gutermann, W. H., and Lisk, D. J., *J. Assoc. Off. Anal. Chem.*, 1977, **60**, 1070.
11. Woodham, D. W., Mitchell, W. G., Loftis, C. D., and Collier, C. W., *J. Agric. Food Chem.*, 1971, **19**, 186.
12. Agemian, H., and Chau, A. S. Y., *Analyst*, 1976, **101**, 732.
13. Johnson, L. G., *J. Assoc. Off. Anal. Chem.*, 1973, **56**, 1503.
14. Cotterill, E. G., *J. Chromatogr.*, 1979, **171**, 478.
15. Draper, W. M., *J. Agric. Food Chem.*, 1982, **30**, 227.
16. Lee, H. B., and Chau, A. S. Y., *J. Assoc. Off. Anal. Chem.*, 1983, **66**, 1023.

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# Preparation of a Chloride-selective Electrode Based on Mercury(I) Chloride - Mercury(II) Sulphide on an Electrically Conductive Epoxy Support

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A chloride-selective electrode based on mercury salts on an electrically conductive epoxy support was prepared and tested. A 1+1 molar mixture of mercury(I) chloride and mercury(II) sulphide was used as the sensor. Response characteristics (linear response range, limit of detection, slope, stability and time of response, pH range and potentiometric selectivity coefficients with respect to bromide, iodide and thiocyanate) were determined. The standards of performance of the electrode were found to be better than those of a commercial electrode with the same type of sensor. Reactions taking place in the membrane are discussed.

**Keywords:** *Chloride-selective electrode; mercury salts; conductive epoxy support*

We have previously reported a simple and easy to implement procedure for the construction of inexpensive "all-solid-state" ion-selective electrodes, in which a layer of very finely powdered sensor is applied to a base of electrically conductive (silver loaded) epoxy resin.[1-4] Using this procedure, electrodes for silver(I) and sulphide,[1,2] halides,[1,3] copper(II)[1,4] and other divalent cations,[1] with silver salts as sensors, were obtained. Their response performances were found to be similar to those of the respective commercial electrodes.[1-4]

This work has been extended to include electrodes based on mercury salts as sensors. This type of sensor was introduced by Sekerka and Lechner,[5-7] who found that an electrode containing a mixture of mercury(II) sulphide and mercury(I) chloride as a sensor showed improved response characteristics to chloride compared with electrodes with a sensor based on a mixture of silver sulphide and silver chloride.[5,8] The lower detection limit of this type of chloride-selective electrode has made its use very convenient for the determination of low levels of chloride in water.[8-13] The performance of a commercial version of the electrode (Graphic Controls PHI 91100) has also been recently evaluated.[14] The use of mixtures of mercury(II) sulphide and mercury(I) chloride as sensors for the self-construction of selective electrodes has presented some difficulties,[10,15] probably owing to the high pressures required to obtain membranes with good mechanical properties.[10] However, Marshall and Midgley[11] successfully applied this type of sensor to the graphite surface of a Růžička Selectrode. The purpose of this study was to investigate the response characteristics of a chloride-selective electrode with this type of sensor, obtained by our procedure for the construction of "all-solid-state" selective electrodes, which does not require high pressures for the preparation of the membrane.

## Experimental

### Apparatus

Potentials were measured with an Orion 811 digital pH meter (reading to ±0.1 mV) and an Orion 605 manual electode switch. Graphs for the determination of response times were obtained with a Radiometer PHM 64 pH meter and a Servograph Rec 61 plotter.

A Philips GAH 110 glass electrode was used for the measurement of pH. Orion 90-02-00 double-junction electrodes (of silver - silver chloride type) were used as reference electrodes (inner filling solution, Orion 90-00-02; outer filling solution, 10% potassium nitrate).

### Reagents

The water used in the preparation of all the standard reagent solutions was de-ionised (Elgastat B114 mixed-bed column unit) and distilled in a quartz still (Heraeus B1 18 double distillation unit). All chemicals were of analytical-reagent grade and were used without further purification. When necessary, stock solutions were standardised by potentiometric titration. Further details are given elsewhere.[14]

### Preparation of the Electrodes

Mercury(II) sulphide (black) was prepared by precipitation, initiated by slowly mixing equal volumes of 0.1 M sodium sulphide and 0.1 M mercury(II) nitrate solutions. The sensor was prepared by thorough grinding of a 1+1 molar mixture of mercury(II) sulphide and mercury(I) chloride (Merck Ref. No. 4425).

The procedure used previously[1] for the preparation of electrodes with silver salt sensors was followed. A piece of silver-loaded commercial epoxy (EPO-TEK 410) was applied to an end of Perspex tube (o.d. 1 cm, length *ca.* 15 cm) to constitute a layer of about 0.7 cm thickness; a shielded cable was fixed to the epoxy inside the tube and, after hardening (at 100 °C for 1 h), a conical cavity was drilled in the epoxy layer. A new piece of epoxy was applied to this cavity and the very thinly powdered sensor was blown against it, while still fresh, from a Pasteur pipette, this operation being repeated several times to obtain a continuous coat of sensor on the epoxy. After hardening (at 80 °C for 4 h), the other end of the tube was closed with Perspex glue. Finally, the sensor layer was polished over glass (Wilks 004–10001) and then with polishing paper (Orion 94-82-01).

### Procedure for the Evaluation of the Electrodes

Standard techniques were used for evaluating the response characteristics of the prepared electrodes. All the measurements were made with the electrodes immersed in solutions kept at 25.0±0.2 °C. Except where otherwise stated the ionic strength of the solutions was adjusted to 0.1 M and the pH to 3 with potassium nitrate and nitric acid.

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The slope, $S$, and standard potential, $E^0$, were obtained from the experimental points in the linear range of calibration by a least-squares adjustment, performed by standard programs of pocket calculators. $R$ is the correlation coefficient of linear regression given by the programs, which measures the goodness of fit.

## Results

### Characteristics of Electrode Response to Chloride

*Reproducibility of preparation*

In order to assess the reproducibility of the preparation procedure and the stability of response (see below) five units were simultaneously calibrated daily with standard solutions of sodium chloride in the range $4 \times 10^{-4}$–$10^{-2}$ M for a period of more than 2 weeks.[2-4] Between calibrations the electrodes were left in de-ionised water, as this was found to be a suitable conditioning medium. (When not in use the electrodes were stored dry and in the dark and before their re-use were polished and conditioned.) Table 1 presents typical results of the calibrations, obtained with one of the units (A). Table 2 gives the average values of the calibration parameters and their standard deviations for the five units (A–E).

The results presented in Table 2 show that the procedure used for electrode construction yields units with reproducible response characteristics.

*Stability of response*

With respect to calibration graphs, the electrodes retain their characteristics over several weeks (Table 1) without any need for restoring the membrane by polishing, provided that they are kept in water between measurements. The reproducibility of repeated calibrations obtained during the same day was generally found to better than 0.5 mV decade$^{-1}$ for the slope and 1 mV for the standard potential (Table 1).

A decrease in slope was normally found after a month or more of use, but the response characteristics could be restored by polishing the membrane with Orion paper followed by conditioning in water. This treatment was found to be very effective even when the membrane had been subjected to strong interferences.

Under normal use, the electrodes do not require frequent polishing and, consequently, although the membrane thickness is small (less than 1 mm) they have high durability. Several units have been used for the determination of chloride in high-purity waters for almost two years and still show good response characteristics. Such durability is a definite advantage over electrodes with the same type of sensors in pressed membranes, which, as mentioned by Tacussel and Fombon[15] and confirmed by ourselves in a previous evaluation of two units of the Graphic Controls electrode,[14] show frequent periods of irregular response and require repeated polishing, which wears out the membrane.

*Lower limit of linear response and limit of detection*

Typical calibrations by the standard additions technique for the determination of these parameters are shown in Fig. 1. Values of *ca.* $10^{-5}$ and *ca.* $5 \times 10^{-6}$ M for the lower limit of linear response and the limit of detection,[16] respectively, were obtained, both being similar to the corresponding values found for a commercial electrode with the same type of sensor.[14] Although less intense than for the commercial electrode, a "memory effect" was found; for example, if the electrode had been immersed in a $10^{-1}$ M chloride solution before calibration, values of *ca.* $10^{-4}$ M were found for the lower limit of linear response. The re-establishment of the normal value above for this parameter requires polishing followed by immersion in water for at least 30 min.

The value for the lower limit of linear response falls within the range of values for the parameter reported in the literature ($2 \times 10^{-6}$–$2 \times 10^{-5}$ M[8,11,14,15]) and extension of the linear range is characteristic of the mercury salt-based chloride electrodes compared with those based on silver salts. A 100-fold increase in linear response range has been reported[5] for pressed membrane electrodes, but previously with a commercial electrode with this type of membrane an approximately 20-fold increase was found[14] as in this work.

An interesting feature shown by the calibration (Fig. 1) is the small variation of potential below the limit of linear

**Table 1.** Stability of the calibration graph* of an electrode unit†,‡

| Time/d | $S$/mV decade$^{-1}$ | $E^\circ$/mV | $R$ | $E(10^{-3})$/mV Calculated | $E(10^{-3})$/mV Read |
|---|---|---|---|---|---|
| 1 | −56.7 | 40.0 | 0.9999 | 210.0 | 210.0 |
| 1 | −57.0 | 39.6 | 0.9999 | 209.5 | 209.3 |
| 2 | −56.3 | 40.2 | 0.9999 | 209.2 | 208.9 |
| 2 | −56.3 | 39.8 | 0.9999 | 208.7 | 208.3 |
| 3 | −55.8 | 40.2 | 0.9999 | 207.4 | 207.3 |
| 3 | −54.9 | 40.0 | 0.9999 | 204.6 | 204.3 |
| 8 | −57.4 | 36.8 | 0.9999 | 209.2 | 209.0 |
| 8 | −57.5 | 36.8 | 0.9999 | 209.2 | 209.2 |
| 9 | −57.4 | 36.6 | 0.9999 | 208.8 | 208.9 |
| 9 | −57.5 | 36.9 | 0.9999 | 209.5 | 209.5 |
| 10 | −57.9 | 35.4 | 0.9999 | 209.0 | 208.9 |
| 10 | −57.3 | 36.0 | 0.9999 | 208.0 | 208.1 |
| 18 | −57.5 | 35.2 | 0.9999 | 207.7 | 207.4 |
| 18 | −57.6 | 34.5 | 0.9999 | 207.4 | 207.5 |

* In the range $4 \times 10^{-4}$–$10^{-2}$ M chloride.
† Unit A in Table 2.
‡ Symbols: $S$ = slope; $E^\circ$ = standard potential (*vs.* S.C.E.); $R$ = correlation coefficient of linear regression; $E(10^{-3})$ = response to $10^{-3}$ M chloride; Calculated, from calibration graph; Read, direct reading.

**Table 2.** Average calibration parameters of several electrode units*,†

| Unit | $S$/ mV decade$^{-1}$ | $E^0$/mV |
|---|---|---|
| A | 56.9 (0.8) | 38 (2) |
| B | 57.8 (0.8) | 33 (2) |
| C | 57.0 (1.0) | 36 (2) |
| D | 56.0 (2.0) | 37 (4) |
| E | 57.0 (2.0) | 37 (4) |

* Averages of 14 calibrations over 18 d, with standard deviations in parentheses.
† For symbols see Table 1.

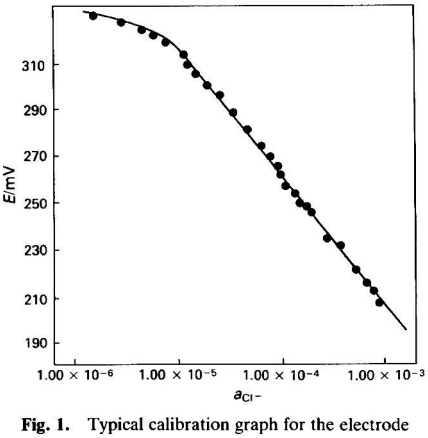


**Fig. 1.** Typical calibration graph for the electrode

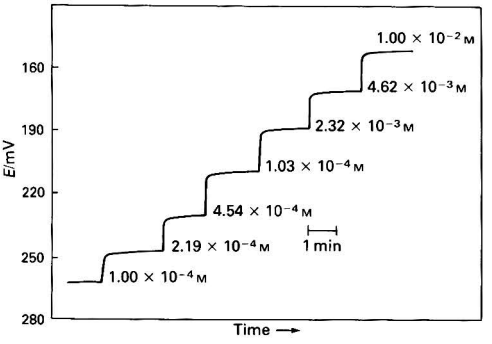


**Fig. 2.** Typical recorder output of dynamic response time determinations for varying concentrations of chloride

response, only about 20 mV in the decade from $10^{-6}$ to $10^{-5}$ M. A similar situation was found by us for the Graphic Controls electrode.[14] In both situations, the potential variation is smaller than that shown by the electrode prepared by Marshall and Midgley[11,12] by coating a Růžička Selectrode with the same type of sensor. As a consequence, in this instance, the practical limit of detection is close to the lower limit of linear detection and the usefulness of the electrode below the latter limit is limited, unless, perhaps, rigorously controlled conditions[12] are applied.

*Slope*

The slope of *ca.* 57 mV decade$^{-1}$ for the units studied (Table 2) was slightly lower than the theoretical value. Although Sekerka and Lechner[5,8] reported a Nernstian response, Tseng and Gutknecht[17] were unable to obtain the same slope for the electrodes prepared by a similar procedure, and obtained slopes of 50–51 mV decade$^{-1}$. However, other workers[10,11,15] have reported slopes in the range 57–59 mV decade$^{-1}$ for electrodes with the same type of sensor constructed by different procedures as was found by us in this and in previous work.[14]

*Standard potential*

Correcting the average value of this parameter for the units included in Table 2, +36 mV, by +242 mV to express S.C.E. against N.H.E. and −7 mV ($S \times \log f_{Cl-}, f_{Cl-} = +0.755$ at $I = 0.1$ M[18]) to compensate for the ionic strength, a value of +271 mV *vs.* N.H.E. was obtained as the standard potential. This value is close to +268 mV, the standard potential of the $Hg_2Cl_2$ - Hg system,[19] suggesting that there is metallic mercury in the membrane to fix $a_{Hg}$ at 1[20,21] (see Discussion).

*Response time*

As shown in Fig. 2, the dynamic response time of the electrode, for increases in chloride concentrations in the range $10^{-4}$–$10^{-2}$ M, is less than 1 min. Sekerka and Lechner[5,8] quoted shorter response times for their electrode than for chloride-selective electrodes with silver salts as sensors, but the present electrode and the corresponding one of the latter type[3] showed similar response times. The electrode prepared by Marshall and Midgley[11] by application of the sensor on a Růžička Selectrode was also found to be slower than the pressed membrane electrode of Sekerka and Lechner.[5,8] Our previous evaluation[14] of the Graphic Controls commercial electrode did not show any improvement of response times over electrodes with silver salts as sensors.

When the electrode is exposed to a decrease in chloride concentration, response times are much longer, especially if the initial value of concentration is high. This "memory effect"

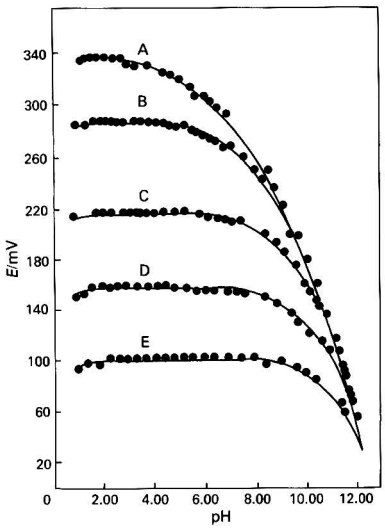


**Fig. 3.** Variation of response potential with pH for various concentrations of chloride: A, $1.00 \times 10^{-5}$ M; B, $1.00 \times 10^{-4}$ M; C, $1.00 \times 10^{-3}$ M; D, $1.00 \times 10^{-2}$ M; and E, $1.00 \times 10^{-1}$ M

(*cf.*, discussion under *Lower limit of linear response and limit of detection*) is much more pronounced for the electrode reported here than for the electrode based on silver salts. This has also been found for the Graphic Controls electrode,[14] based on a pressed membrane sensor, and also for the electrode of Marshall and Midgley.[11] As the degree of compactness of the sensor in the membrane is different for these three electrodes, the effect cannot be ascribed only to surface irregularities. A possible explanation is a tendency for adsorption of chloride at the membrane surface, which may be related to the high values of the stability constants of $[HgCl_n]^{(n-2)-}$ complexes. Indeed such values are much higher than those of the corresponding $[AgCl]^{(n-1)-}$ species.[22] It was observed that when this electrode was immersed in water for conditioning, its potential suffered a quick change until a stable value was reached, which may be due to the washing out of chloride from the surface of the membrane.

**Effect of pH on the Response**

The influence of the pH on the response of the electrode at fixed chloride concentrations between $10^{-1}$ and $10^{-5}$ M (in solutions with an ionic strength adjusted to 0.2 M with potassium nitrate) is presented in Fig. 3. The potential is independent of pH from a lower limit of pH between 1.5 and 2 and an upper limit that depends much more markedly on the chloride concentration than for the hydroxide interference in other solid membrane electrodes, *e.g.*, for chloride-selective electrodes with silver salts as sensors.[3] The marked variation of the upper limit of the operative response plateau can be understood when the value of $K_{Cl,OH} = a_{Cl-}/a_{OH-}$ is calculated from the solubility products of $Hg_2Cl_2$ and $Hg_2O$,[14] the large value obtained (*ca.* $3 \times 10^2$) explaining the observed variation. This feature of the chloride-selective electrode with an $Hg_2Cl_2$ - HgS sensor has not been discussed in the literature, where a value of pH 6 is invariably indicated as the upper limit of operation.[5,15,23] These results show that this value of pH is too high for the measurement of chloride at low concentrations (less than *ca.* $10^{-4}$ M) and support the procedure established by Marshall and Midgley[11] where the hydrogen ion concentration is fixed at a constant value of $10^{-2}$ M.

**Table 3.** Potentiometric selectivity coefficients, $K^{Pot}_{Cl,X}$

| | This work* at chloride concentration | | | Reference | | | |
|---|---|---|---|---|---|---|---|
| X | $10^{-3}$ M | $10^{-4}$ M | Calculated† | 5 | 15 | 14‡ | 23 |
| $SCN^-$ | 70 ± 12 | 7 ± 1 | 40 | 2 | – | ~25 | – |
| $Br^-$ | 70 ± 11 | 43 ± 6 | $2 \times 10^4$ | $6.3 \times 10^2$ | ~$10^2$ | ~25 | $6 \times 10^2$ |
| $I^-$ | 30 ± 9 | 5 ± 1 | $3 \times 10^{10}$ | $3.2 \times 10^3$ | ~10 | ~25 | $3 \times 10^3$ |

* Values are averages of six results (duplicate determinations with three units) obtained at the chloride concentrations given.
† Calculated[14] by $K^{Pot}_{Cl,X} = K_{SO}(Hg_2Cl_2) - K_{SO}(Hg_2X_2)$ using values of $K_{so}$ given in reference 27.
‡ Obtained at a $10^{-4}$ M chloride concentration.

A comparison of Fig. 3 with similar results for the Graphic Controls electrode[14] shows that this electrode is more sensitive to pH changes below 2, which may be explained by the sensor being less compact when on the epoxy support than in a pressed membrane, where solubilisation is more difficult.

### Interferences

For this type of electrode, there are discrepancies between literature values[5,14,15,23] for the potentiometric selectivity coefficients, relative to interferences of anions whose mercury(I) salts are more insoluble than mercury(I) chloride (namely bromide, iodide and thiocyanate), as well as anomalies in their relative values found by some workers.[14,15] In this study, the coefficients were determined by the mixed solution method (with the chloride concentration fixed at $10^{-3}$ and $10^{-4}$ M, without adjustment of the ionic strength but with the pH fixed at *ca.* 4 M with nitric acid). The results of replicate determinations showed a certain degree of variability owing to the lack of reproducibility of the straight segments corresponding to response to interferences, even when the experimental conditions (rate of interferent addition, criterion of readings, etc.) were kept constant. Therefore, the experimental values presented in Table 3, which are averages of the results of six determinations, should be considered only as orders of magnitude of the parameter. In Table 3, literature values are also included for comparison, as well as values obtained from the solubility products, which were calculated using standard procedures.

The experimental values of the selectivity coefficients obtained for the electrode on a conductive epoxy support were much lower than those predicted by calculations. This result is similar to that found with electrodes with silver salts,[1,3] but the differences between the experimental and calculated values appear to be more accentuated in this instance. Moreover, as found by Tacussel and Fombom[15] for the bromide and iodide interferences, the relative strength of interferences found follows the order $SCN^- \geqslant Br^- \geqslant I^-$, which is opposite to the order predicted from calculations. In our previous study of the Graphic Controls electrode, which for interferences was less detailed than this one, about the same value was obtained for the selectivity coefficients with respect to the three interferents.[14] Tacussel and Fombom[15] have suggested that such anomalies have kinetic causes.

Another interesting point shown by these results is that the values of the selectivity coefficients at the $10^{-4}$ M chloride level are smaller than the corresponding values at $10^{-3}$ M. The electrode seems to feel the effect of these interferences less extensively at lower concentrations, and this may also be a consequence of slower response to lower concentrations of interferents. However, it should be pointed out that in these experiments, in contrast to observations in similar experiments with chloride-selective elctrodes with silver salts as sensors,[3] visual inspection of the membrane did not show any strange precipitates on its surface. As the chemical behaviour of an $Hg_2Cl_2$ - HgS membrane seems to be extremely complex (see Discussion), an interference mechanism more complex than that accepted for electrodes with silver salts as sensors cannot be ruled out.

### Response to the Mercury(I) Ion

Calibrations for the response to mercury(I) ion were obtained by the titration technique using $10^{-2}$–$10^{-4}$ M mercury(I) nitrate solutions. These showed a slope slightly higher than the theoretical one, 32.0 (0.5) mV decade$^{-1}$ and a standard potential of 819 (2) mV *vs.* N.H.E. These are average values obtained from four determinations with three units, the standard deviations being given in parentheses. The standard potential includes a correction of +13 mV for ionic strength compensation, calculated as before with $f_{Hg^{2+}} = 0.355$ at $I = 0.1$ M.[18] The value of the standard potential is similar to that found previously[14] for the Graphic Controls electrode (813 mV), both being greater than the standard potential of the $Hg^{2+}$ - Hg system (792 mV).[19] The difference between the electrode standard potential and the standard potential of the relevant couple is larger in this instance than when the response to chloride is considered.

## Discussion

Our previous work on ion-selective electrodes based on mixtures of silver salts of divalent metal sulphides and silver(I) sulphide on a conductive epoxy support has shown that this construction procedure yields electrodes with response characteristics similar to those of the corresponding commercial electrodes.[1–4] There is indirect evidence that, in these electrodes, the metallic silver in the epoxy support does not contact the solution.[3,4,24] This is also suggested by observation of the surface of the membranes by scanning electron microscopy, which shows that in the small resin areas (*ca.* $10^{-3}$–$10^{-4}$ mm$^2$) exposed between sensor microcrystals, the silver signal is very weak (less than 1% of the signal of microcrystals and probably having this origin).[25]

In this situation the operation of the constructed electrode was found to be less troublesome than for the commercial electrode evaluated previously,[14] even though the numerical values of the characteristic parameters of electrode response are very similar. Less frequent polishing for maintaining electrode performance was required and sudden outbreaks of bad behaviour of the electrode were not observed as for the Graphic Controls electrode.[14] Other problems of the chloride and other selective electrode with pressed membranes based on mercury salts, *e.g.*, irreproducibility of response[15] or troublesome operation,[26] have been discussed in the literature.[10,15,17,26] The proposed method of construction minimises such problems.

The values of the standard potentials of this electrode in response to chloride (+271 mV) and mercury(I) ions (+819 mV) are, respectively, close to the values for the $Hg_2Cl_2$ - Hg and $Hg^{2+}$ - Hg couples and yield a value of $2.8 \times 10^{-19}$ for the solubility product of $Hg_2Cl_2$, in agreement with literature values,[27] *e.g.*, 1.3 × 10–18 M[3]. These data show that the electrode responds to chloride as a second kind electrode.

According to Koebel[20] and Buck and Shepard,[21] this requires the occurrence of free mercury in the membrane to fix the value of the activity of the metal equal to unity.

The presence of the free metal in the sensor is understood if the value of the equilibrium constant for the disproportionation of $Hg_2Cl_2$ (s = solid, sol = solution)

$$Hg_2Cl_2(s) + S^{2-}(sol) = Hg(l) + HgS(s) + 2Cl^-(sol) \quad (1)$$

is considered. It can be calculated from the solubility products of $Hg_2Cl_2$ ($1.3 \times 10^{-18}$) and HgS (black) ($1.6 \times 10^{-52}$)[27] and the equilibrium constant of the disproportionation ($K_{disp}$)

$$Hg_2^{2+}(sol) = Hg(l) + Hg^{2+}(sol) \quad .. \quad (2)$$

by the expression

$$K = [Cl^-]^2/[S^{2-}] = K_{disp} \times K_{so}(Hg_2Cl_2)/K_{so}(HgS) \quad (3)$$

$K_{disp}$ can be calculated from the standard potentials of the couples $Hg^{2+} - Hg_2^{2+}$ (+907 mV) and $Hg_2^{2+} - Hg^0$ (+792 mV),[19] to be $K_{disp} = 10^{-1.95}$. A value of *ca.* $10^{32}$ is obtained for $K$. This value is so large that the reaction can occur even for large, although reasonable, values of chloride concentration in solution. Sulphide, provided by the intrinsic solubility of the mercury(II) sulphide, is used up, *i.e.*, the reaction (1) occurs instead of the dissolution of this salt.

Alternatively, if the intrinsic solubility of $Hg_2Cl_2$ is considered [or when the electrode is immersed in a solution of mercury(I) ion], precipitation of free mercury at the membrane surface is explained by the reaction

$$Hg_2^{2+}(sol) + S^{2-}(sol) = Hg(l) + HgS(s) \quad .. \quad (4)$$

of which the equilibrium constant

$$K' = 1/([Hg_2^{2+}] \times [S^{2-}]) = K_{disp}/K_{so}(HgS) \quad .. \quad (5)$$

is also very large ($K' \approx 10^{50}$).

These calculations show that the disproportionation of mercury(I) ion in the membrane is spontaneous in the thermodynamic sense and is expected to occur indefinitely with conversion of $Hg_2Cl_2$ into HgS (and free mercury) with the release of chloride into the solution. It is interesting to observe that Hulanicki *et al.*,[26] in a paper discussing the construction of a bromide-selective electrode based on mercury salts, where reactions similar to those above are involved,[28] reported the appearance of small droplets of metallic mercury on the walls of the die where a membrane consisting of $Hg_2Cl_2$, HgS and $Ag_2S$ had been pressed with thermal treatment.

This thermodynamic instability may explain the problems connected with the troublesome operation observed for the chloride and other electrodes with pressed membranes based on mercury salts.[10,14,15,17,26] A practically stable response of such electrodes requires that a metastable equilibrium state be reached in the membrane. The results of this work and their comparison with previous[14] and literature results, suggest that the use of an unpressed mixture makes the attainment of such a metastable state easier than when the mixture is disturbed by pressure and heating.

Conditioning of the mercury salt electrodes in water after polishing the final stage of the construction or when the membrane is regenerated has been recommended[5,13,14,23] and was found to be necessary in this work in order to obtain acceptable response characteristics. The effectiveness of such conditioning may result from the removal of chloride and other soluble species formed by the disproportionation reactions (discussed above) from the membrane surface.

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## References

1. Lima, J. L. F. C., and Machado, A. A. S. C., *in* Albaiges, J., *Editor*, "Analytical Techniques in Environmental Chemistry," Volume 2, Pergamon Press, Oxford, 1982, p. 419.
2. Lima, J. L. F. C., and Machado, A. A. S. C., *Rev. Port. Quim.*, 1979, **21**, 15.
3. Lima, J. L. F. C., and Machado, A. A. S. C., *Rev. Port. Quim.*, 1979, **21**, 153.
4. Lima, J. L. F. C., and Machado, A. A. S. C., *Rev. Port. Quim.*, 1982, **24**, 156.
5. Lechner, J. F., and Sekerka, I., *J. Electroanal. Chem.*, 1974, **57**, 317.
6. Sekerka, I., and Lechner, J. F., *J. Electroanal. Chem.*, 1976, **69**, 339.
7. Sekerka, I., and Lechner, J. F., *Anal. Lett.*, 1976, **9**, 1099.
8. Sekerka, I., Lechner, J. F., and Wales, R., *Water Res.*, 1975, **9**, 663.
9. Sekerka, I., Lechner, J. F., and Harrison, L., *J. Assoc. Off. Anal. Chem.*, 1977, **60**, 625
10. Bailey, P., Wilson, J., Karpel, S., and Riley, M., *in* Pungor, E., *Editor*, "Conference on Ion Selective Electrodes, Budapest, 1977," Elsevier, Amsterdam, 1978, p. 201.
11. Marshall, G. B., and Midgley, D., *Analyst*, 1978, **103**, 438.
12. Marshall, G. B., and Midgley, D., *Analyst*, 1979, **104**, 55.
13. Ryan, T. E., Peterson, A. J., and Subsara, W. P., *in* Moody, G. J., *Editor*, "International Symposium on Electroanalysis in Clinical, Environmental and Pharmaceutical Chemistry," UWIST, Cardiff, 1981, paper 5.
14. Lima, J. L. F. C., and Machado, A. A. S. C., *Rev. Port. Quim.*, 1982, **24**, 61.
15. Tacussel, J., and Fombom, J. J., *in* Pungor, E., *Editor*, "Conference on Ion Selective Electrodes, Budapest, 1977," Elsevier, Amsterdam, 1978, p. 567.
16. Guilbault, G. G., *Editor*, "Recommendations for Publishing Manuscripts on Ion-Selective Electrodes," *Pure Appl. Chem.*, 1981, **53**, 1907.
17. Tseng, P. K. C., and Gutknecht, W., *Anal. Chem.*, 1976, **48**, 1996.
18. Kielland, J., *J. Am. Chem. Soc.*, 1937, **59**, 1675.
19. Lurie, J., "Handbook of Analytical Chemistry," Mir, Moscow, 1975, p. 305.
20. Koebel, M., *Anal. Chem.*, 1974, **46**, 1559.
21. Buck, R. P., and Shepard, V. R., *Anal. Chem.*, 1974, **46**, 2097.
22. Sillen, L. G., and Martell, A. G., *Editors*, "Stability Constants of Metal Ion Complexes," Special Publication No. 17, Chemical Society, London, 1964, pp. 286–288 and 292–293.
23. "Ultra-Sensitive Solid State Chloride Electrode PHI 91100 (Instruction Manual)," Graphic Controls, Buffalo, NY, USA.
24. da Silva, M. G. P., Lima, J. L. F. C., and Machado, A. A. S. C., *Port. Electrochim. Acta*, 1984, **2**, 29.
25. Lima, J. L. F. C., Machado, A. A. S. C., and Sá, C. M., "Abstracts of the Sixth Meeting of the Sociedade Portuguesa de Química," Sociedada Portuguesa de Química, Aveiro, 1983, paper PC36.
26. Hulanicki, A., Lewandowski, R., and Lewenstam, A., *Anal. Chim. Acta*, 1979, **110**, 197.
27. Lurie, J., "Handbook of Analytical Chemistry," Mir, Moscow, 1975, p. 110
28. Lima, J. L. F. C., and Machado, A. A. S. C., *Port. Electrochim. Acta*, submitted for publication.

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# Reduction in Size by Electrochemical Pre-treatment at High Negative Potentials of the Background Currents Obtained at Negative Potentials at Glassy Carbon Electrodes and its Application in the Reductive Flow Injection Amperometric Determination of Nitrofurantoin

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The reduction of dissolved molecular oxygen at a glassy carbon electrode was shown to be made more difficult on electrochemically pre-treating a newly polished glassy carbon disc electrode (3 mm in diameter) in 0.1 M sulphuric acid solution at −2.7 V for 1 min. By this means a background current of only 1 μA was obtained when this electrode was held at −1.05 V in a flow injection system incorporating extensive PTFE transmission tubing and using a deoxygenated pH 7 Britton - Robinson buffer as eluent (flow-rate 6.5 ml $min^{-1}$). The size of the signal obtained when 100 μl of eluent that had not been deoxygenated were injected as the sample blank was only 0.02 μA at −0.7 V. When nitrofurantoin was determined using these latter conditions, this blank signal was equivalent to about $2 \times 10^{-7}$ M nitrofurantoin.

**Keywords**: *Electrochemical pre-treatment; amperometric detection; reduction; flow injection analysis; nitrofurantoin determination*

Increasing attention is being paid to the advantages of electrochemically pre-treating glassy carbon electrodes used for amperometric detection in HPLC and in flow injection analysis.[1–8] The studies reported to date have been made to improve the performance of glassy carbon electrodes used for monitoring oxidation processes at positive potentials. In many irreversible oxidation processes, electrochemical pre-treatment first at a high positive potential and then at about −1.0 V reduces the overpotential for oxidation of the determinand such that an improved hydrodynamic voltammogram is obtained. Oxidation occurs more completely and at a less positive potential such that a higher and more reproducible signal is obtained. At any particular potential the background signal is also increased, but this does not detract significantly from the technique.

An important HPLC method that involves reductive amperometric detection at a glassy carbon electrode held at negative potentials is the determination of vitamin K and its analogues.[9,10] Hart *et al.*[10] determined vitamin $K_1$ in a 95% methanol eluent that was 0.05 M in a pH 3 sodium acetate - acetic acid electrolyte holding the potential of the glassy carbon electrode at −1.0 V; the eluent was deoxygenated with nitrogen and an all-metal solvent delivery system was used to prevent the re-entry of oxygen. Calibration graphs were obtained by injecting 1–10 ng of vitamin $K_1$. Adsorbed product on the electrode was removed periodically by holding the electrode at +0.7 V, which re-oxidised the product as the reduction process is quasi-reversible. Hanging mercury drop electrodes have been used by other workers, notably by Lloyd[11–13] for determining explosives residues, as detectors of reductive processes. The rigorous exclusion of oxygen has been an important feature of all of these methods.

In the work described in this paper a study was made of the possibility of improving signals for reductive processes at glassy carbon electrodes held at negative potentials by applying electrochemical pre-treatment.

## Experimental

Flow injection analysis was carried out in a single-channel system that has been described previously.[14] Eluent flow was produced by means of an Ismatec Mini-S peristaltic pump. Sample (approximately 100 μl) was injected with a Rheodyne 5020 low-pressure injection valve connected to a laboratory-built detector cell by means of 50 cm of 0.58 mm bore PTFE tubing. The detector cell holds the glassy carbon electrode only, eluent being presented to it in a wall-jet configuration. The cell is used partially immersed in an electrolyte having the same composition as the eluent. A counter platinum and a conventional potentiometric calomel reference electrode are placed in the electrolyte to obtain electrical contact with the working electrode. The glassy carbon disc electrode (3 mm diameter) was constructed from Le Carbonne glassy carbon and was mounted in PTFE. An eluent flow-rate of 6.5 ml $min^{-1}$ was used. The potential of the glassy carbon electrode was controlled by means of a PAR 174 polarographic analyser and current signals were monitored on a Linseis L650 $y$ - $t$ recorder. Linear sweep voltammetry was carried out at a sweep rate of 10 mV $s^{-1}$ using the same working, counter and reference electrodes immersed in the appropriate measuring solution.

### Preliminary Linear Sweep Experiments in a Static System

During studies of the effect of positive- and negative-potential electrochemical pre-treatments of glassy carbon electrodes on oxidation processes at low positive potentials, it was noticed that electrochemical pre-treatment at high negative potentials was effective in making smaller the background currents obtained at negative potentials. This is clearly illustrated in Fig. 1, in which base-line linear sweep voltammograms obtained with a static electrode system in 0.01 M sulphuric acid before and after electrochemical pre-treatment are shown; the electrode was electrochemically pre-treated in 0.1 M sulphuric acid. Pre-treatment at −3 V in the static mode is seen to remove the oxygen reduction wave most effectively. Further, electrochemical pre-treatment was shown to be effective only when carried out in dilute sulphuric acid; attempts to effect pre-treatment in Britton - Robinson buffer solution of pH between 2 and 8 were unsuccessful. The pre-treatment that had been effected in dilute sulphuric acid, however, was also as effective when the electrode was used in these buffer solutions.

Linear sweep voltammograms obtained for the reduction of

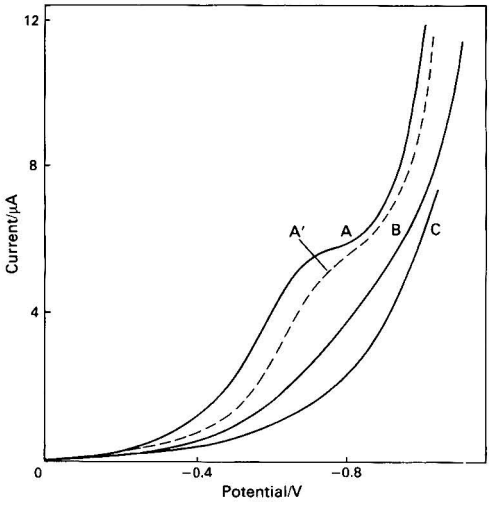


**Fig. 1.** Blank linear sweep voltammograms in 0.01 M sulphuric acid without deoxygenating the solution. A and A', first and second scans at a newly polished glassy carbon electrode; B, scan after pre-treatment at −2.5 V for 1 min in 0.1 M sulphuric acid; and C, scan after pre-treatment at −3 V for 1 min in 0.1 M sulphuric acid

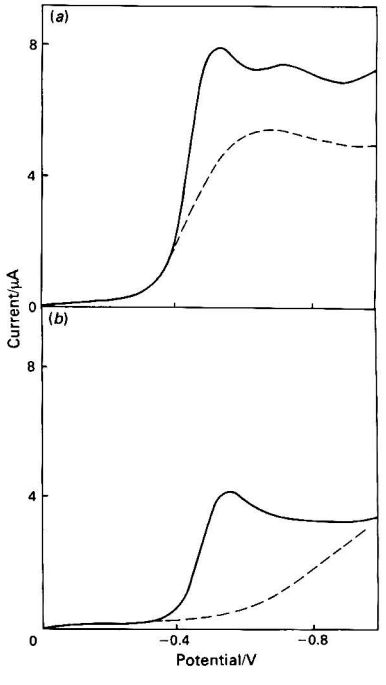


**Fig. 2.** Linear sweep voltammograms of nitrofurantoin ($2 \times 10^{-4}$ M) in undeoxygenated pH 7 Britton - Robinson buffer. (*a*) At a newly polished glassy carbon electrode; and (*b*) at an electrode pre-treated at −3 V for 1 min. The blank linear sweep voltammograms are given as broken lines in both instances

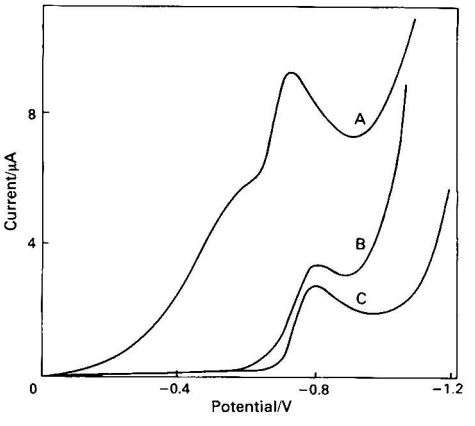


**Fig. 3.** Linear sweep voltammograms of cephalonium (100 μg ml$^{-1}$) in 0.1 M sulphuric acid. A, Without deoxygenating the solution at a newly polished glassy carbon electrode; B, without deoxygenating the solution at an electrode pre-treated at −3 V for 1 min; and C, after deoxygenating the solution at a newly polished glassy carbon electrode

nitrofurantoin, which occurs at about −0.58 V in pH 7 Britton - Robinson buffer, are shown in Fig. 2. When the electrode is newly polished a hump due to the reduction of dissolved molecular oxygen is apparent as a post-peak. After pre-treating the electrode at −3 V this hump is no longer apparent. An illustration of the oxygen reduction process occurring before that of a determinand is shown in Fig. 3, in which linear sweep voltammograms for the reduction of the cephalosporin cephalonium are shown. Here also electrochemical pre-treatment at −3 V removes visible signs of the oxygen reduction process. Polarographic methods are available for the determination of nitrofurantoin[15] and cephalonium.[16]

### Effect of Electrochemical Pre-treatment at High Negative Potentials in Flow Injection Analysis

In using a glassy carbon electrode for amperometric detection in HPLC or flow injection analysis, two characteristics of the system should be considered before the quality of the signal obtained with the determinand is studied. These are the background current associated with the eluent and the blank signal obtained when a control blank is injected. When the eluent is used as the control blank, clearly eluent and sample are the same and no signal should be observed when the control blank is injected, except at high sensitivities owing to disturbance to the flow of eluent caused by the process of injecting the eluent. In determinations made at potentials where oxygen reduction occurs, however, a finite blank signal will be observed if the oxygen contents of the eluent and the blank sample solution differ. A negative signal will be observed if the eluent contains more oxygen than the blank sample. Clearly analytical determinations become very unreliable when the level of interferent in the solvent system and sample solution have to be balanced, and this is particularly so with dissolved molecular oxygen. In general, with increasing background current the detection limit attainable is increased; with the system used in this work it has generally been observed that if the background current reaches 1 μA then determinations can only be made down to about $5 \times 10^{-6}$ M and that this also applies at positive potentials where the background current is not associated with the reduction of oxygen.

The results reported here for electrochemically pre-treated electrodes were obtained with electrodes pre-treated either at

−2.7 V for 1 min in a static system before being inserted into the detector cell or at −3 V for 1 min on-line in 0.1 M sulphuric acid at a flow-rate of 2 ml $min^{-1}$. These were found to be the optimum off-line and on-line electrochemical pre-treatment conditions. This latter process was readily effected by switching eluents before the pump. The use of higher pre-treatment potentials than those recommended led to higher background noise. The background current levels obtained at various potentials with the flow injection system in which pH 7 Britton - Robinson buffer was used as the eluent are shown in Fig. 4. These were obtained for a newly polished electrode and for pre-treated electrodes in all instances with and without deoxygenation of the eluent with nitrogen (it should be borne in mind that the term "deoxygenation," which is used extensively by polarographers, is misleading in that the oxygen concentration is reduced only to a particular level that is determined by the effectiveness of the "deoxygenation" process and also by the effectiveness of preventing oxygen from re-entering the eluent before the measurement is made). It is clear from Fig. 4 that electrochemical pre-treatment extends the useful range of the electrode to more negative potentials both when the eluent is deoxygenated and when it is not and that the static electrochemical pre-treatment process is more effective than the on-line pre-treatment. In effect, on pre-treatment the overpotential for the reduction of oxygen at the glassy carbon electrode is being increased, *i.e.*, the reduction of oxygen is being made more difficult by the pre-treatment process.

Perhaps not surprisingly, electrochemical pre-treatment has a more significant effect on the useful range of the electrode when the oxygen content of the eluent has been reduced to a lower level by deoxygenation. Nevertheless, deoxygenation of eluent and sample solutions is a time-consuming task and there is a distinct advantage to be gained in avoiding the necessity of having to carry it out. Compounds such as vitamin $K_3$, which can be determined at potentials less negative than −0.5 V, can be determined at low levels even with an unpre-treated electrode without having to deoxygenate the eluent and sample solutions. Nevertheless, even in these instances the background current is reduced and the detection limit should be lowered by using a pre-treated electrode. Electrochemical pre-treatment produces a slight extension of the useful range of the electrode in an eluent that has not been deoxygenated and this should allow other compounds to be determined without the need to deoxygenate the eluent or sample solutions, particularly if determinations are to be made at high concentrations.

The extension of the useful range on pre-treating the glassy carbon electrode, however, is much greater for the deoxygenated eluent. From Fig. 4 it can be seen that the potential at which a background current of 1 μA is obtained is moved from −0.72 to −1.05 V on pre-treating the electrode at −2.7 V in the static mode. Hence electrochemical pre-treatment should make amperometric detection possible for compounds that are reduced (or oxidised) at these more negative potentials. Again, the added advantage that lower background currents are obtained in determining compounds at lower negative potentials should not be overlooked.

Deoxygenation of eluent in a flow injection system by means of nitrogen is readily carried out, and nitrogen can be bubbled continuously through the eluent in the eluent reservoir during determinations with no great inconvenience or loss of time once the initial deoxygenation has been effected. Deoxygenation of every sample solution is extremely time consuming, however, and the need to do this should be avoided if at all possible. The size of signals obtained at various potentials on injecting pH 7 Britton - Robinson buffer that had not been deoxygenated into deoxygenated eluent of the same composition is illustrated in Fig. 5. These results were obtained with both newly polished and electrochemically pre-treated electrodes. The marked effect of electrochemical

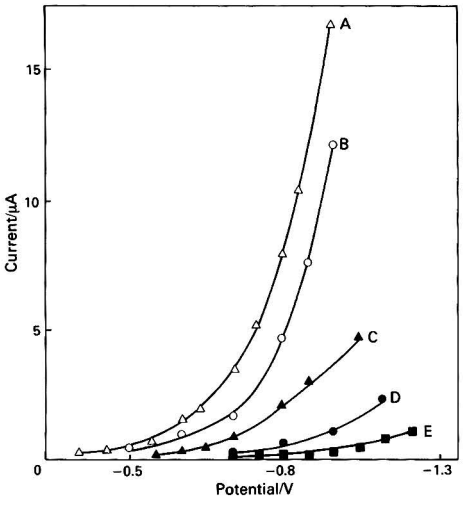


**Fig. 4.** Background currents obtained with flow injection amperometry using pH 7 Britton - Robinson buffer as eluent. A, Without deoxygenating the solution at a newly polished glassy carbon electrode; B, without deoxygenating the solution at an electrode pre-treated at −3 V for 1 min on-line; C, after deoxygenating the solution at a newly polished glassy carbon electrode; D, after deoxygenating the solution at an electrode pre-treated at −3 V for 1 min on-line; and E, after deoxygenating the solution at an electrode pre-treated at −2.7 V for 1 min (in a static system)

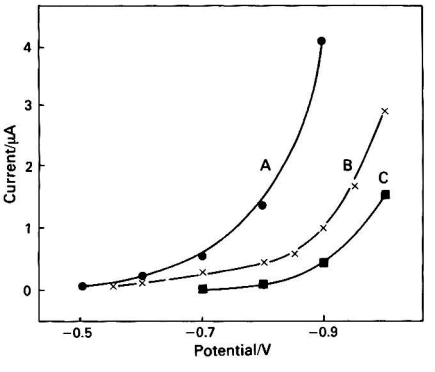


**Fig. 5.** Blank hydrodynamic voltammograms representing the size of signals obtained on injecting undeoxygenated eluent into deoxygenated eluent (pH 7 Britton - Robinson buffer). A, At a newly polished electrode; B, at an electrode pre-treated on-line in 0.1 M sulphuric acid at −3 V for 1 min; and C, at an electrode pre-treated in the static mode at −2.7 V for 1 min

pre-treatment on the size of the signal obtained can be clearly seen. The potential at which the blank signal due to oxygen in the blank sample reaches 1 μA is moved from −0.77 to −0.95 V on pre-treating the electrode at −2.7 V in the static mode.

In Figs. 6 and 7 are shown hydrodynamic voltammograms of nitrofurantoin in pH 7 Britton - Robinson buffer using newly polished and pre-treated electrodes, respectively. In both instances the hydrodynamic voltammograms that are shown were obtained using deoxygenated eluent. The effect of deoxygenating the sample on the hydrodynamic voltammograms obtained is also clear. Blank hydrodynamic voltammograms in which undeoxygenated eluent was injected into

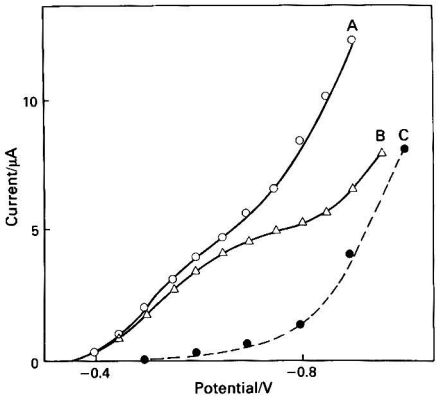


**Fig. 6.** Hydrodynamic voltammograms obtained at a newly polished electrode for injection of nitrofurantoin ($2 \times 10^{-4}$ M) into deoxygenated pH 7 Britton - Robinson buffer. A, Sample solution undeoxygenated; B, sample solution deoxygenated; and C, undeoxygenated blank injection

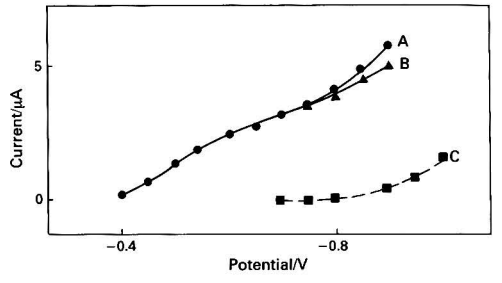


**Fig. 7.** Hydrodynamic voltammograms obtained at an electrode pre-treated in the static mode at −2.7 V for 1 min for injection of nitrofurantoin ($2 \times 10^{-4}$ M) into deoxygenated pH 7 Britton - Robinson buffer. A, Sample solution undeoxygenated; B, sample solution deoxygenated; and C, undeoxygenated blank injection

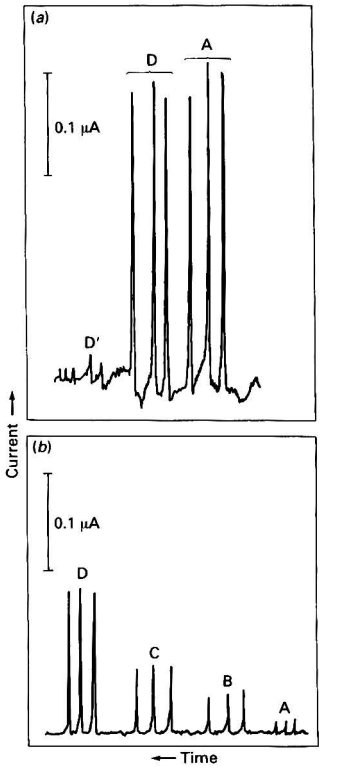


**Fig. 8.** Signals obtained near the determination limit for nitrofurantoin at (*a*) a newly polished electrode and (*b*) at an electrode pre-treated at −2.7 V in the static mode for 1 min. Measurement potential = −0.65 V. Nitrofurantoin concentration: A, 0; B, $2 \times 10^{-7}$ M; C, $5 \times 10^{-7}$ M; and D and D', $10 \times 10^{-7}$ M. Eluent and sample solution D', deoxygenated; sample solutions, A–D, undeoxygenated

deoxygenated eluent are also shown. It should be noted that the signals due to the reduction of nitrofurantoin are made smaller by the pre-treatment process. Clearly, the reduction of nitrofurantoin is also being inhibited, although not to the same extent as the reduction of oxygen. The precision of the signals, however, remained excellent. The beneficial effect of the electrochemical pre-treatment in making smaller the size of the oxygen signal can be clearly seen in Fig. 7. At the current sensitivity used in obtaining the hydrodynamic voltammograms shown in Fig. 7 there is no difference in the signal at −0.7 V on deoxygenating the sample solution. Hence it is clear that at these levels of determinand there is no need to deoxygenate the sample solutions.

Fig. 8 shows signals obtained near the determination limit both with a newly polished electrode and a pre-treated electrode. The measurement potential used here was −0.65 V to reduce the oxygen blank to an acceptable level for this concentration of determinand. The large-scale removal of background noise on electrochemically pre-treating the electrode can be seen clearly. The extensive reduction in the signal from the oxygen dissolved in the sample solution on electrochemical pre-treatment is also apparent; this blank is equivalent to $6 \times 10^{-8}$ M nitrofurantoin. At significantly higher concentrations determinations would normally be made at −0.7 V where the blank signal is equivalent to $2 \times 10^{-7}$ M nitrofurantoin. At levels of nitrofurantoin above $5 \times 10^{-6}$ M coefficients of variation for five injections at the same concentration were typically less than 1%.

## Conclusions

Electrochemical pre-treatment at high negative potentials in dilute sulphuric acid is effective in inhibiting the reduction of dissolved molecular oxygen at glassy carbon electrodes and therefore lowers the background currents caused by reduction of dissolved oxygen when such electrodes are used at negative potentials in flow injection analysis and, by extrapolation, in HPLC applications. With nitrofurantoin a slight loss of determinand signal also occurs, but without loss of precision. For most reversible systems it is expected that little or no loss of signal would be experienced, *i.e.*, that the systems would remain reversible. It is further expected that detection limits even for compounds that are determined at low negative potentials, where oxygen is not a major interferent at high determinand concentrations, will be lowered. Further studies are being made on such systems.

In flow injection applications using PTFE transmission tubing it is possible to deoxygenate eluents to a sufficiently low level to enable compounds that are reduced at potentials up to about −0.7 V to be determined at a pre-treated electrode without the need to deoxygenate the sample solutions.

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## References


1. Blaedel, W. J., and Jenkins, R. A., *Anal. Chem.*, 1975, **47**, 1337.
2. Chan, H. K., and Fogg, A. G., *Anal. Chim. Acta*, 1979, **111**, 281.
3. Van Rooijen, H. W., and Poppe, H., *Anal. Chim. Acta*, 1981, **130**, 9.
4. Engstrom, R. C., *Anal. Chem.*, 1982, **54**, 2310.
5. Ravinchandran, K., and Baldwin, R. P., *Anal. Chem.*, 1983, **55**, 1782.
6. Engstrom, R. C., and Strasser, V. A., *Anal. Chem.*, 1984, **56**, 136.
7. Fogg, A. G., Fernández-Arciniega, M. A., and Alonso, R. M., *Analyst*, 1985, **110**, 851.
8. Fogg, A. G., Fernández-Arciniega, M. A., and Alonso, R. M., *Analyst*, 1985, **110**, 1201.
9. Ikenoya, S., Abe, K., Tsuda, T., Yamano, Y., Hiroshima, O., Ohmae, M., and Kawabe, K., *Chem. Pharm. Bull.*, 1979, **27**, 1237.
10. Hart, J. P., Shearer, M. J., McCarthy, P. T., and Rahim, S., *Analyst*, 1984, **109**, 477.
11. Lloyd, J. B. F., *J. Chromatogr.*, 1983, **256**, 323.
12. Lloyd, J. B. F., *J. Chromatogr.*, 1983, **257**, 227.
13. Lloyd, J. B. F., *J. Chromatogr.*, 1983, **261**, 391.
14. Fogg, A. G., and Summan, A. M., *Analyst*, 1984, **109**, 1029.
15. "United States Pharmacopeia," Twentieth Revision, United States Pharmacopeial Convention, Rockville, MD, 1980, p. 549.
16. Fogg, A. G., Fayad, N. M., Burgess, C., and McGlynn, A., *Anal. Chim. Acta*, 1979, **108**, 205.


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# Determination of Vitamin C by Flow Injection Analysis

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A photometric procedure for the determination of vitamin C using chloramine T by two flow injection analysis (FIA) methods is described. The first is in the presence of potassium iodide - starch solution using the normal FIA technique (linear determination range: 15–150 μg ml$^{-1}$, RSD ±0.97% and sampling frequency 90 samples h$^{-1}$) and the second is in the presence of potassium bromide - methyl red solution using an FIA titration (linear determination range: 0.5–1000 μg ml$^{-1}$, RSD ±0.82% and a sampling frequency of 30 samples h$^{-1}$). The two methods are compared with each other and with a conventional titration procedure.

**Keywords:** *Vitamin C determination; flow injection analysis; potassium iodide - starch; potassium bromide - methyl red*

Numerous conventional methods have been described for the determination of vitamin C with electroanalytical detection. most frequently used type of detection. Methods based on the use of Ti(III),[1] Ce(IV),[2] hexacyanoferrate(III),[3] bromine cyanide,[4] vanadium,[5] iodate,[6] 2,4-dinitrophenylhydrazine[7,8] and α,α′-dipyridyl[9–11] have been described, each with their advantages and disadvantages.

Flow injection analysis (FIA) has been applied twice to the determination of vitamin C with electro analytical detection. The first contribution was reported by Strohl and Curran,[12] who used a reticulated glassy carbon electrode to carry out the coulometric and amperometric determination of the vitamin, achieving detection limits of a few nanograms. The major advantage of the coulometric method is that the analyte is completely electrolysed and can be determined without calibration graphs. However, the sampling frequency is decreased with respect to the amperometric method. The second contribution was an amperometric method using immobilised enzyme (ascorbic acid oxidase) reactors that allowed the determination of the vitamin in brain tissue, with good results.[13]

In order to develop a photometric - FIA method for the determination of vitamin C we used the reagent chloramine T in the presence of starch - potassium iodide solution or methyl red - potassium bromide solution as an indicator,[14] by normal FIA in the first instance and by an FIA titration without a gradient chamber (expansion-scale technique)[15–18] in the second. Both methods are compared with each other and with the conventional titration procedures.

## Experimental and Results

### Apparatus

An FIA-5020 Tecator Analyzer, an SP6-500 Pye Unicam UV - visible spectrophotometer and a 178.12 QS Hellma flow cell (inner volume 18 μl) were used.

### Reagents

*Chloramine T solution*, 5 × 10$^{-4}$ M.

*Starch - potassium iodide solution.* Containing 5.25 g of soluble starch and 0.50 g of potassium iodide in 500 ml of distilled water.

*Methyl red - potassium bromide solution.* Containing 0.5 ml of methyl red, 0.1% in ethanol, made up to 100 ml with 0.5% KBr solution.

*Ascorbic acid solutions.* Prepared by direct weighing.

All solutions were stable for at least 24 h.

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### Determination of Vitamin C with Chloramine T and Starch - Potassium Iodide Solution

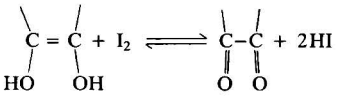
Although this determination is commonly carried out by titration[14] this is unsuitable for use with FIA when starch is employed as an indicator, due to the fleeting appearance of the blue colour of the starch - iodine complex in the reactor. A plateau is not obtained when a high sample volume is injected because the excess of chloramine T degrades the starch - iodine complex. It is therefore essential that the blue colour should have developed by the time that the sample passes through the detector.

*Manifold and chemical system*

The manifold used is shown in Fig. 1. The design is very simple. The sample, dissolved in an acidic medium, is injected into the chloramine T stream after merging with a starch - iodide stream. Iodide forms HI in an acidic medium, which subsequently reacts with chloramine T:

$$MeC_6H_4SO_2NNaCl + 2HI \rightleftharpoons MeC_6H_4SO_2NH_2 + NaCl + I_2$$

The iodine formed oxidises vitamin C:



Once vitamin C has been fully consumed, iodine binds to starch, which consitutes the indicator reaction.

Sulphuric acid is added directly to the sample as insertion into the chloramine T stream results in the precipitation of the reagent. When the sample only contains sulphuric acid (blank) no iodine is consumed because of the absence of vitamin C and thus the maximum signal is obtained. The presence of vitamin C causes a weakening of the analytical signal proportional to its concentration.

*Optimisation of variables*

The optimum values for the FIA variables (Fig. 1) are as follows: flow-rate ($q$) = 2.04 ml min$^{-1}$; injected volume ($V_i$) = 96.2 μl; reactor length ($L$) = 55 cm; inner diameter of the reactor ($\phi$) = 0.5 mm. These values allow the blue colour of the starch - iodine complex to be formed as the sample plug passes through the detector, where it is monitored at 650 nm. The optimisation of the variables ensures the maximum difference between the reference and the sample peaks.

The influence of the chloramine T concentration is one of the most important variables in this determination. Its effect can be observed in Fig. 2. For high concentrations of chloramine T the presence of vitamin C has no effect on the

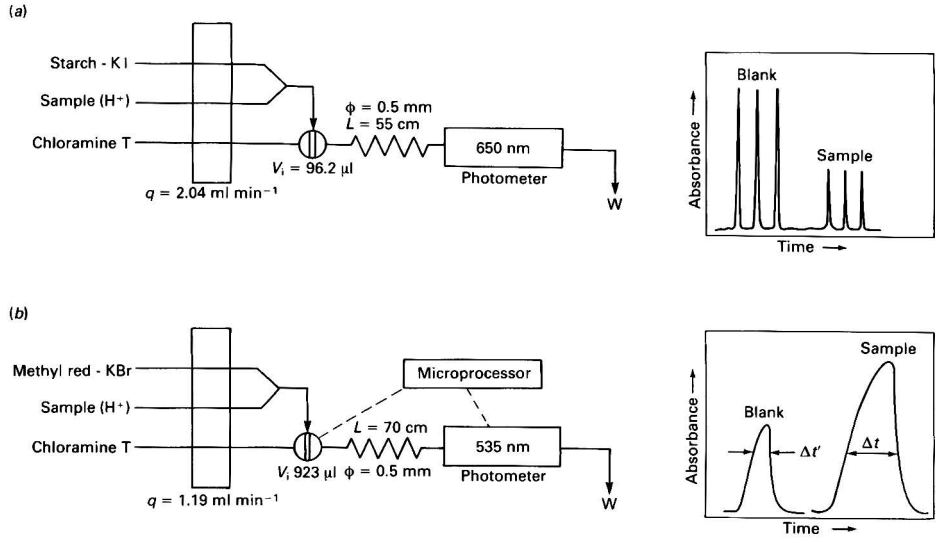



**Fig. 1.** Manifolds used in the determination of vitamin C and recordings obtained by: (*a*) normal FIA mode and (*b*) FIA titration (for details see, text)

analytical signal. The maximum difference between the blank and the sample signal is obtained with $5 \times 10^{-4}$ M chloramine T solutions. For lower concentrations of the amine the difference in absorbance decreases owing to the drastic weakening of the blank signal.

The pH of the sample is adjusted by the addition of $H_2SO_4$. The reaction medium must be acidic to allow the formation of HI. The difference in absorbance between the blank and the sample is high at low pH, but above a sulphuric acid concentration of 0.75 M the increase in this difference is small and some peak broadening is observed. A 0.90 M sulphuric acid concentration was chosen as the optimum. The most suitable concentrations of KI and starch are indicated under Experimental.

It is well known that an increase in temperature is a negative factor for the development of the blue colour of the starch - iodine complex; therefore, the experiments were performed at room temperature.

*Determination of vitamin C*

This method allows the determination of vitamin C according to the equation:

$$\Delta A = 0.0035\ [\text{vitamin C}] - 0.0053;\ r = 0.999$$

where $\Delta A$ is the difference in the absorbance between the blank and the sample containing vitamin C (whose concentration is expressed in µg ml$^{-1}$). The equation is satisfied between 15 and 150 µg ml$^{-1}$, the RSD ($P = 0.05$) for the determination of 100 µg ml$^{-1}$ of vitamin C being ±0.97%. The sampling frequency is 90 samples h$^{-1}$.

## Determination of Vitamin C with Chloramine T and Methyl Red - Potassium Bromide Solution

This method, based on the measurement of the peak width at a pre-determined height from the base line, has been termed "high-speed titrations" by Ramsing *et al.*[15] and "scale-expansion techniques" by Stewart and Rosenfeld[16] following the suggestions from Pardue and Fields.[17,18]

The chemical system is similar to the one described above but the indicator is methyl red - potassium bromide solution.

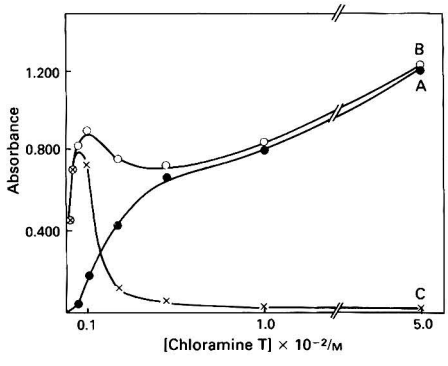



**Fig. 2.** Influence of the chloramine T concentration in the stream. Absorbance values obtained with: A, sample with $5 \times 10^{-3}$ M vitamin C; B, blank; and C, difference between sample and blank

This system may be used for FIA titrations because the red colour of methyl red in an acidic medium allows a wide peak to be obtained when a large volume of this indicator is injected.

*Manifold and chemical system*

The manifold used is similar to the one described above. In this instance the methyl red - potassium bromide stream is substituted for the starch - potassium iodide one. The HBr formed by merging this stream with that of the sample in an acidic medium ($H_2SO_4$) is oxidised by chloramine T and the $Br_2$ produced oxidises vitamin C to dehydroascorbic acid. An excess of bromine with respect to the stoicheiometric amount of vitamin C present reacts with methyl red, causing its degradation. The presence of the vitamin in the sample (resulting in a decrease in the degradation of the indicator) brings about a widening of the peak obtained at 535 nm. The measurement of the peak width (in seconds) at a pre-determined absorbance value is the basis of the FIA titrations.

**Table 1.** Calibration graphs for the determination of vitamin C by FIA titration

| Absorbance* | Range/M | Equation | r |
|---|---|---|---|
| 0.020 | $(0.3–1.7) \times 10^{-5}$ | $\Delta t = 8.248 \log [\text{vitamin C}] + 49.63$ | 0.991 |
| | $(0.1–2.8) \times 10^{-3}$ | $\Delta t = 18.17 \log [\text{vitamin C}] + 102.94$ | 0.991 |
| 0.050 | $(0.3–1.7) \times 10^{-5}$ | $\Delta t = 4.67 \log [\text{vitamin C}] + 28.55$ | 0.993 |
| | $(0.2–1.1) \times 10^{-4}$ | $\Delta t = 15.18 \log [\text{vitamin C}] + 79.08$ | 0.997 |
| | $(0.2–5.7) \times 10^{-3}$ | $\Delta t = 20.60 \log [\text{vitamin C}] + 100.38$ | 0.991 |
| 0.100 | $(0.3–1.7) \times 10^{-5}$ | $\Delta t = 4.46 \log [\text{vitamin C}] + 28.01$ | 0.990 |
| | $(0.02–5.7) \times 10^{-3}$ | $\Delta t = 15.88 \log [\text{vitamin C}] + 80.86$ | 0.999 |

*Absorbance at which the peak width ($\Delta t$) is measured

**Table 2.** Determination of vitamin C in synthetic samples

| [Vitamin C] by normal FIA*/M | | | [Vitamin C] by FIA titration †/M | | |
|---|---|---|---|---|---|
| Added | Found | Error, % | Added | Found | Error, % |
| $9.02 \times 10^{-5}$ | $9.66 \times 10^{-5}$ | +6.8 | $2.08 \times 10^{-5}$ | $2.17 \times 10^{-5}$ | +4.3 |
| $1.70 \times 10^{-4}$ | $1.77 \times 10^{-4}$ | +4.1 | $5.68 \times 10^{-5}$ | $5.82 \times 10^{-5}$ | +2.5 |
| $2.84 \times 10^{-4}$ | $2.67 \times 10^{-4}$ | −5.9 | $1.70 \times 10^{-4}$ | $1.71 \times 10^{-4}$ | +0.6 |
| $5.66 \times 10^{-4}$ | $5.56 \times 10^{-4}$ | −1.7 | $4.26 \times 10^{-4}$ | $4.23 \times 10^{-4}$ | −0.7 |
| $1.14 \times 10^{-3}$ | $1.17 \times 10^{-3}$ | +2.6 | $8.52 \times 10^{-4}$ | $8.63 \times 10^{-4}$ | +1.3 |
| $1.42 \times 10^{-3}$ | $1.43 \times 10^{-3}$ | −1.4 | $1.42 \times 10^{-3}$ | $1.39 \times 10^{-3}$ | −2.1 |

* Equation: $\Delta A = 615$ [vitamin C] − 0.005
† Equation $\Delta t = 15.882 \log$ [vitamin C] + 80.86

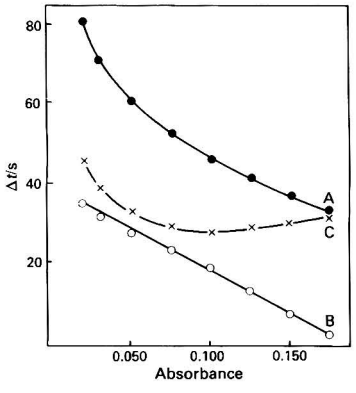



**Fig. 3.** Variation of $\Delta t$ with the value of the absorbance at which the measurement of the peak width is performed. A, Sample with $5 \times 10^{-3}$ M vitamin C; B, blank; C, difference between sample and blank

*Optimisation of variables*

The optimum values for the FIA variables are as follows: $q = 1.19$ ml min$^{-1}$; $V_i = 923.0$ µl; $L = 70$ cm and $\phi = 0.5$ mm. The large sample volume injected ensures that wide peaks are obtained, the lower the vitamin C concentration, the wider the peaks. This sample volume, which is unusually large for normal FIA, is the key to FIA titrations.

The concentrations of some reagents are decisive. Thus, the optimum concentration of chloramine T is $5 \times 10^{-4}$ M, which allows maximum differences to be obtained between the sample and the blank. The samples contain 1% of concentrated $H_2SO_4$ (0.15 M).

Owing to the short reactor length, the temperature does not exert a significant influence. Thus, the experiments were performed at room temperature (18–20 °C).

The absorbance at which the peak width is measured influences the sensitivity of the determination. It is advisable to measure the peak width at the absorbance yielding the maximum difference between the sample and the blank. Fig. 3 shows this effect for a sample containing 100 µg ml$^{-1}$ of vitamin C. The differences increase as the absorbance approaches zero, but owing to instrumental limitations, peak widths over 99.9 s cannot be measured. The increase in peak width observed above 0.100 A (curve C, Fig. 3) is not significant, and as the corresponding peak widths are very small, the errors introduced in these measurements are large. Therefore, the criteria adopted to run the calibration graphs at different absorbances according to the high or low concentration of vitamin C in the samples are as follows: the peak width is measured at 0.100 A for high and at 0.020 or 0.050 for low analyte concentrations.

*Calibration graphs*

A plot of the peak width ($\Delta t$) *versus* logarithm of the unknown concentration, characteristic of these titrations, shows different linear ranges according to the value of the absorbance at which $\Delta t$ is measured (as is shown in Table 1). In each instance the contribution of the blank has been subtracted. This represents a major improvement of the method proposed above; a wider range and lower limit of detection are attained, possibly owing to the instability of the starch - iodine complex in the presence of chloramine T, which was a limiting factor in the previous method. An RSD ($P = 0.05$) of ±0.82% and a sampling frequency of 30 h$^{-1}$ were obtained in the determination of 100 µg ml$^{-1}$ of vitamin C ($\Delta t$ measured at 0.050 A).

**Study of Interferents in Each Method**

In a study of the interferences in both FIA methods, $Fe^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $NH_4^+$, $PO_4^{3-}$, $CO_3^{2-}$, oxalate, glucose, glycine, hystidine, urea, cysteine and uric acid can all be tolerated at a ten-fold excess over vitamin C, except for cysteine and uric acid, which interfere when present at the same concentration as the vitamin in the FIA titration method. It is worth noting the smaller influence of foreign species in the FIA technique compared with conventional methods.[14] This behaviour was observed in earlier studies[19] and is attributable to the non-equilibrium state of the chemical system at the point of detection. The higher selectivity of the method employing iodine is a result of the lower oxidising strength of this halogen

compared with bromine. Likewise, the higher acidity of the sample in the first method facilitates the precipitation and removal of interferents (*e.g.*, uric acid).

### Comparison of the Results Obtained by the Two Methods

The results obtained by both methods for the determination of vitamin C in several synthetic samples are given in Table 2. A similar degree of accuracy is afforded by both methods.

The determination of vitamin C with chloramine T by FIA titration offers the following advantages over the normal FIA technique: a wider determination range; a higher sensitivity; and a slightly higher accuracy.

These advantages stem from the high instability of the starch - iodine complex in the presence of chloramine T, which is a limiting factor in the determination of vitamin C by the normal FIA technique. Normal FIA does however have some advantages, such as the need for only one calibration graph, smaller sample volume, higher selectivity, which makes it applicable to the determination of vitamin C in urine, and higher sampling rate.

The determination of vitamin C by FIA titration presents the following advantages over the manual procedure[14]: it is faster and simpler, as the colour change in the manual method is slow and difficult to detect visually; it uses reagent and sample more sparingly; the manipulation and intervention of the operator is a minimum; and it is more sensitive and accurate.

## References


1. Gupta, D., Sharma, P. D., and Gupta, Y. K., *Talanta,* 1975, **22**, 913.
2. Rao, G. G., and Sastri, G. S., *Anal. Chim. Acta*, 1971, **56**, 325.
3. Sastri, G. S., and Rao, G. G. *Talanta*, 1972, **19**, 212.
4. Paul, R. C., Chauhan, R. K., and Prakash, J., *Indian J. Chem.*, 1971, **9**, 879.
5. Eremina, Z. I., and Gurevich, V. G., *Zh. Anal. Khim.*, 1964, **19**, 519.
6. Murty, C. N., and Bapat, N. G., *Fresenius Z. Anal. Chem.*, 1963, **199**, 367.
7. Roe, J. H., Oesterling, M. B., and Llamron, C. M., *J. Biol, Chem.*, 1948, **174**, 201.
8. Teruuchi, J., and Kitazato, K., *Arch. Exp. Med.*, 1951, **1**, 55.
9. Zannoni, U., Lynch, M., Goldstein, S., and Sato, P., *Biochem. Med.*, 1974, **11**, 41.
10. Okamura, M., *Clin. Chim. Acta*, 1980, **108**, 259.
11. Okamura, M., *Bitamin*, 1981, **55**, 495.
12. Strohl, A. N., and Curran, D. J., *Anal. Chem.*, 1979, **51**, 1045.
13. Bradberry, C. W., and Adams, R. N., *Anal. Chem.*, 1983, **55**, 2439.
14. Verma, K. K., and Gulati, A. K., *Anal. Chem.*, 1980, **52**, 2336.
15. Ramsing, A., Růžička, J., and Hansen, E. H., *Anal. Chim. Acta,* 1981, **129**, 1.
16. Stewart, K. K., and Rosenfeld, A. G., *Anal. Chem.,* 1982, **54**, 2368.
17. Pardue, H. L., and Fields, B., *Anal. Chim. Acta*, 1981, **124**, 39.
18. Pardue, H. L., and Fields, B., *Anal. Chim. Acta*, 1981, **124**, 65.
19. Linares, P., Luque de Castro, M. D., and Valcárcel, M., *Anal. Lett.*, 1985, **18 B1**, 67.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# Determination of Vitamin C in Urine by Flow Injection Analysis*

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A spectrophotometric method with flow injection analysis is described for the determination of vitamin C in urine, which presents substantial improvements over the existing conventional manual and automatic methods. Determinations can be carried out at the μg ml$^{-1}$ level; the method has an average recovery error of ±2.5% and a sampling frequency of 90 samples h$^{-1}$ can be attained.

**Keywords:** *Vitamin C determination; flow injection analysis; urine*

The determination of vitamin C in its three forms (ascorbic acid, AA; dehydroascorbic acid, DHAA; and 2,3-diketogulonic acid, DKGA) has been carried out in numerous ways.[1] The most frequent method is based on the titration of ascorbic acid with 2,6-dichlorophenolindophenol, 2,6-DCPIP[2,3]; nevertheless, this method is only useful for samples with small amounts of foreign species as it is subject to numerous interferences from reducing species such as sulphur dioxide, tannins, cysteine, sulphydryl compounds, certain metal ions, plant pigments, reductants and similar compounds. For this reason, depending on the sample matrix, this method and others that eliminate possible interferents have been used so far. For the determination of vitamin C in urine and blood the method most commonly used is that of Roe and Kuether,[4] which is based on the formation of coloured osazones in a concentrated sulphuric acid medium, via the reaction between 2,4-dinitrophenylhydrazine and 2,3-diketogulonic acid, an oxidised form of ascorbic acid. Nevertheless, in spite of overcoming most interferences this procedure does have several disadvantages: it is complex and time consuming (over 180 min per analysis are required); and it is subject to other interferents such as hexoses, pentoses, glucuronic acid, reductants, hystidine and several other amino acids, although most of them do not interfere at the normal level in which they are normally found in urine and blood. To improve this method several modifications have been suggested: the use of different oxidising agents such as bromine,[5] 2,6-DCPIP[6] and benzoquinone[7]; different reaction conditions[8]; the use of different acids[6]; or the use of different separative techniques.[5] Automatic methods using reagents such as 2,6-DCPIP,[9] *o*-phenylenediamine (DPD)[10] and 2,4-dinitrophenol (2,4-DNP)[11,12] have also been developed with the aid of Technicon AutoAnalyzers. These methods are the most similar to that proposed in this paper, in which the chloramine T - KI - starch reaction and flow injection analysis (FIA) are used jointly. This latter development is an easily automatable technique,[13] which, in addition, is inexpensive. These facets, together with the absence of interferents in the method, make this a good alternative to routine vitamin C analysis.

## Experimental

All reagents and apparatus used in this work were the same as described previously,[14] except for a Metrohm Dosimat E535 automatic burette. The FIA manifold employed has also been described previously.[14]

### Procedure

Urine samples were obtained from individuals who had been given a pharmaceutical compound containing 0.5 g of vitamin C. Samples were collected in a polyethylene flask to which a final concentration of 500 mg l$^{-1}$ of oxalic acid had been added. The urine samples were subsequently diluted 1 + 5 with 0.9 M $H_2SO_4$ and pumped to their confluence with a KI - starch stream until they filled the loop of the injection valve, which was inserted into the chloramine T stream. The concentrations of vitamin C in urine shown in Tables 1 and 3 were obtained with diluted samples.

## Results and Discussion

Firstly the two methods proposed in reference 14 were applied to the determination of vitamin C in urine; however, the FIA titration method (in which KBr - methyl red solution is used as the indicator) is subject to major interferences owing to its low selectivity. Urine samples with a concentration of vitamin C of 60 μg ml$^{-1}$, as determined by the normal FIA method, provided concentrations over 6000 μg ml$^{-1}$ with the FIA titration technique. This result was predictable as sulphydryl compounds interfere at the same analyte level because of the strong oxidising character of $Br_2$, a product yielded in the indicator reaction (chloramine T - KBr) which acts as an oxidant for vitamin C. Conversely, the normal FIA method, which uses KI - starch solution as the indicator, provided good results and was therefore chosen for the application of the method to real samples.

### Stability of the Samples

The study was performed on urine samples diluted 1 + 5 with 0.9 M $H_2SO_4$. Oxalic acid, a reducing agent, was used as a preservative.[15] Concentrations between 0 and 2000 μg ml$^{-1}$ were used in this study. For concentrations of oxalic acid equal to or higher than 500 μg ml$^{-1}$, the analytical signal yielded by the sample remained constant for at least 24 h, whilst in the absence of a preservative a decrease in the signal of 10% was observed during the same time interval. A concentration of 500 μg ml$^{-1}$ was therefore chosen for subsequent experiments.

The reproducibility of the method was studied at two analyte concentrations (30.30 and 65.70 μg ml$^{-1}$) with nine different samples in each instance and at two different times (1.0 and 3.5 h). The values obtained for the relative standard deviation (r.s.d.) were as follows. After 1 h: 30.30 μg ml$^{-1}$ (r.s.d. = ±0.93%); 65.70 μg ml$^{-1}$ (r.s.d. = ±0.32%). After 3.5 h: 30.30 μg ml$^{-1}$ (r.s.d. = ±0.90%); 65.70 μg ml$^{-1}$ (r.s.d. = ±0.30%). The precision was good in both instances but was improved by increasing the analyte concentration.

For the study of the recovery of vitamin C in urine, six samples were taken at different times from an individual who had taken 0.5 g of vitamin C. Concentrations of 20, 40 and 80 μg ml$^{-1}$ of vitamin C were added to each sample. The results obtained are listed in Table 1; a good recovery was observed (the average recovery = 99.6% with an average error of 2.24%). These data indicate the absence of interferents in this

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**Table 1.** Results for the recovery of vitamin C in urine

| Urine sample | Vitamin C found in urine diluted 1 + 5/ μg ml$^{-1}$ | Vitamin C added/μg ml$^{-1}$ 20.00 Found/μg ml$^{-1}$ | 20.00 Recovery, % | 40.00 Found/μg ml$^{-1}$ | 40.00 Recovery, % | 80.00 Found/μg ml$^{-1}$ | 80.00 Recovery, % |
|---|---|---|---|---|---|---|---|
| 1 | 20.97 | 40.06 | 95.5 | 60.00 | 97.6 | 102.00 | 102.0 |
| 2 | 29.03 | 49.68 | 103.2 | 69.68 | 101.6 | 109.02 | 100.0 |
| 3 | 36.13 | 56.13 | 100.0 | 74.84 | 96.8 | 115.00 | 98.6 |
| 4 | 39.35 | 59.68 | 101.6 | 78.71 | 98.4 | 119.50 | 100.2 |
| 5 | 41.61 | 61.95 | 101.6 | 80.65 | 97.6 | 119.50 | 97.4 |
| 6 | 59.35 | 78.71 | 96.8 | 102.00 | 106.6 | 137.50 | 97.7 |

**Table 2.** Comparison between the FIA and conventional titration methods for the determination in vitamin C in synthetic samples

| Amount of vitamin C added/μg ml$^{-1}$ | FIA method Amount found/μg ml$^{-1}$ | FIA method Relative error, % | Titration method Amount found/μg ml$^{-1}$ | Titration method Relative error, % | Error of FIA method relative to titration method, % |
|---|---|---|---|---|---|
| 20.00 | 19.04 | −4.8 | 21.54 | 5.7 | −11.6 |
| 40.00 | 43.79 | 9.5 | 41.57 | 3.9 | 5.3 |
| 50.00 | 49.56 | −0.9 | 50.73 | 1.5 | −2.3 |
| 60.00 | 56.35 | −6.1 | 62.70 | 4.5 | −10.1 |
| 70.00 | 68.64 | −1.9 | 73.97 | 5.7 | −7.2 |
| 80.00 | 79.78 | −0.3 | 85.25 | 6.5 | −6.4 |
| 100.00 | 106.34 | 6.3 | 106.38 | 6.4 | 0.0 |
| 120.00 | 120.53 | 0.4 | 126.11 | 5.1 | −4.4 |
| 140.00 | 135.98 | −2.9 | 145.13 | 3.6 | −6.3 |
| Mean error, % | | 3.7 | | 4.8 | 4.6 |

**Table 3.** Comparison between the FIA and conventional titration methods for the determination of vitamin C in urine samples (diluted 1 + 5) and containing 9.26 μg ml$^{-1}$ of vitamin C

| Amount of vitamin C added/μg ml$^{-1}$ | FIA method Amount found/μg ml$^{-1}$ | FIA method Relative error, % | Titration method Amount found/μg ml$^{-1}$ | Titration method Relative error, % | Error of FIA method relative to titration method, % |
|---|---|---|---|---|---|
| 10.00 | 21.07 | 9.4 | 21.84 | 13.4 | −3.5 |
| 30.00 | 39.26 | 0.0 | 41.57 | 5.9 | −5.5 |
| 40.00 | 47.85 | −2.9 | 51.43 | 4.4 | −6.7 |
| 50.00 | 59.88 | 1.0 | 61.29 | 3.4 | −2.3 |
| 60.00 | 71.01 | 2.5 | 69.57 | 0.4 | 2.1 |
| 70.00 | 78.60 | −0.8 | 79.61 | 0.4 | −1.3 |
| 90.00 | 102.69 | 3.5 | 95.81 | −3.5 | 7.2 |
| 110.00 | 119.43 | 0.1 | 114.34 | −3.7 | 4.0 |
| 130.00 | 136.29 | −2.2 | 134.56 | −3.4 | 1.3 |
| Mean error, % | | 2.5 | | 4.3 | 3.8 |

method, which is attributable to the weak oxidising character of the $I_2$ formed in the reaction.

## Comparison of Methods

The FIA method used in this work was compared with the conventional titration technique[16] (Fig. 1) for synthetic samples and urine samples. In addition, both methods were compared with the determination of vitamin C by direct weighing.

### *Analysis of synthetic samples*

On analysing nine different samples by the two methods average errors of 3.7 and 4.8% were obtained by the FIA and conventional titration methods, respectively, both relative to direct weighing (Table 2). In the conventional titration procedure the errors were always positive, possibly owing to the difficulty in determining the end-point, as it is not based on a colour change, but in the persistence of the colour. Moreover, the kinetics of the reaction are very slow in the vicinity of the equivalence point, so that it is necessary to wait for a certain time after each addition of titrant before making the measurements. This shortcoming is not present in the FIA

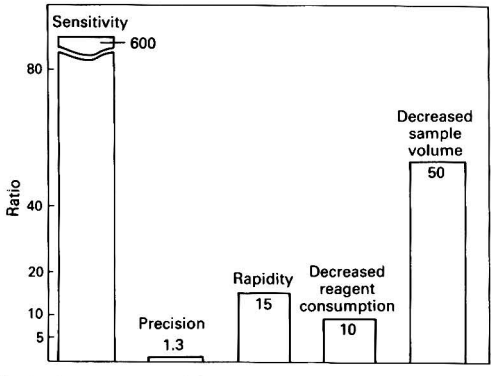


**Fig. 1.** Improvements of the suggested procedure over the conventional titration procedure

method, which affords a sampling frequency of 90 samples h$^{-1}$ compared with 6 samples h$^{-1}$ in the conventional titration. In addition, the titration technique requires the use of an

automatic burette (reading to 0.01 ml) owing to the small reagent volume needed.

*Analysis of urine samples*

The same study performed on urine samples with final concentrations of 9.26 μg $ml^{-1}$ after a 1 + 5 dilution (Table 3) yielded average errors of 2.7 and 4.3% for the FIA and conventional titration methods, respectively, both relative to direct weighing. These errors are slightly smaller than those obtained for synthetic samples. The average error of the FIA method relative to the conventional method is 3.8% in this instance.

These results reveal a smaller error with the proposed method compared with the conventional method, for both synthetic and urine samples.

When the proposed method is compared with automatic methods such as that of Pelletier and Brassard[12] it can be concluded that the proposed method has several advantages over the latter: although the latter technique offers higher sensitivity and similar precision and recovery, the working scheme is very complex (13 channels are necessary as opposed to 3 in the FIA method); maintenance is expensive and time consuming (it requires daily washing with 75 ml of $HNO_3$ and distilled water for 40 min); the flow cell must be washed weekly with dichromate solution and the tubing system must be changed after 120 determinations, whilst the FIA manifold only requires washing for 5 min with distilled water after a working day and allows up to 10000 determinations to be performed (90 determinations $h^{-1}$, 8 h a day, for 14 d) before changing the tubing system. In addition, the maximum sampling rate achievable is 13 samples $h^{-1}$ (with the use of a sampler) compared with 90 samples $h^{-1}$ for the suggested method.

## References


1. Cooke, J. R., and Moxon, R. E. D., *in* Counsell, J. N., and Hornig, D. H., *Editors*, "Vitamin C," Applied Science, Barking, 1981, pp. 167–198.
2. Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 303.
3. Birch, T. W., *Biochem. J.*, 1933, **27**, 590.
4. Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1943, **147**, 399.
5. Zloch, Z., and Ginter, E., *Z. Klin. Chem.*, 1970, **8**, 302.
6. Pelletier, O., *J. Lab. Clin. Med.*, 1968, **72**, 674.
7. Saari, J. C., *Anal. Biochem.*, 1966, **15**, 537.
8. Roe, J. H., *J. Biol. Chem.*, 1961, **236**, 1611.
9. Egberg, D. C., *J. Sci. Food Agric.*, 1973, **24**, 789.
10. Dunmire, D. L., *J. Assoc. Off. Anal. Chem.*, 1979, **62**, 648.
11. Aeschbacker, H. U., and Brown, R. G., *Clin. Chem.*, 1972, **18**, 965.
12. Pelletier, O., and Brassard, R., *Adv. Autom. Anal. Technicon Int. Congr.*, 1973, **9**, 73.
13. Válcarcel, M., and Luque de Castro, M. D., "Flow Injection Analysis: Principles and Applications," Ellis Horwood, Chichester, in the press.
14. Lázaro, F., Ríos, A., Luque de Castro, M. D., and Válcarcel, M., *Analyst*, 1986, **111**, 163.
15. Haddad, P., *J. Chem. Educ.*, 1977, **54**, 192.
16. Verma, K. K., and Gulati, A. K., *Anal. Chem.*, 1980, **55**, 1045.


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# Flow Injection - Hydride Generation System for the Determination of Arsenic by Molecular Emission Cavity Analysis

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A system is described that permits the simple and rapid determination of nanogram amounts of arsenic (0.1–10 μg ml$^{-1}$) in microlitre volumes of sample solutions by flow injection and hydride generation coupled with molecular emission cavity analysis. The effect of some interfering ions, including Ni, Ag, Zn, Cu, Te and Se, was eliminated or minimised by using a 0.01 M EDTA - 0.2 M sodium iodide - 1.5 M hydrochloric acid carrier solution in which the sample was injected. The method permits a sampling rate of *ca.* 100 measurements per hour. The method is illustrated by the determination of arsenic in NBS orchard leaves reference material with good accuracy.

**Keywords:** *Arsenic determination; hydride generation; flow injection analysis; molecular emission cavity analysis*

There is much published work on the determination of arsenic in different kinds of matrices.[1–4] In addition to photometric methods,[5–8] hydride generation in conjunction with atomic absorption spectrometry (AAS),[9–11] inductively coupled plasma atomic emission spectrometry (ICP-AES),[12,13] atomic fluorescence spectrometry[14,15] and molecular emission spectrometry[16] are widely used for the determination of arsenic. Conventional molecular emission cavity analysis (MECA)[17] and hydride generation in conjunction with MECA[18–21] have also been applied to the determination of nanogram amounts of arsenic.

Flow injection analysis (FIA)[22] has been applied together with amperometry and voltammetry for the determination of arsenic at μg ml$^{-1}$ levels.[23–25] Astrom[26] and Liversage and Van Loon[27] introduced the hydride generation approach combined with FIA for the determination of bismuth and arsenic, respectively. By using their procedure, nanogram amounts of both elements were determined with good accuracy and up to 200 measurements per hour were possible.[27]

FIA has also recently been used in combination with MECA for the determination of nanogram amounts of sulphur[28] and phosphorus-containing[29,30] compounds. A combination of FIA and hydride generation with MECA detection has not been reported before. This paper describes the advantages of such a combination. The determination of arsenic is based on the generation of arsine in an FIA system followed by its separation from the liquid phase and transport with argon to the MECA cavity, where the AsO emission intensity is measured at 400 nm. The method has been applied successfully to the determination of arsenic in NBS orchard leaves standard reference material.

## Experimental

### Apparatus

The configuration of the experimental system used is shown in Fig. 1. The flow injection system consisted of a five-channel peristaltic pump (Sage, Orion Research) and a rotary injection valve (Rheodyne Model 7125), to which a loop of given volume was attached. All tubing was made of Tygon (0.5 mm i.d.), except the side-tube, which connected the MECA cavity to the FIA system, made of stainless steel (0.5 mm i.d.) as previously described.[28] The confluence T-joints and connections were made of Perspex.

The instrument for MECA measurements, the cavity holder support device and the circular emission burner were as previously described.[28,29] A water-cooled steel MECA cavity, which was similar to that described earlier,[28] was used. However, in addition a small hole was made in the side-wall of the cavity (Fig. 2) in order to introduce a flow of oxygen. The introduction of oxygen is necessary in order to restrict the emission within the oxy-cavity.[17]

The gas - liquid separator, which was essentially the same as that described by Vijan *et al.*,[31] was miniaturised as far as possible using tubing of 2.0 mm i.d.

### Reagents

All reagents used were of analytical-reagent grade, unless stated otherwise. De-ionised, doubly distilled water was used throughout.

*Arsenic stock solution,* 1000 μg ml$^{-1}$. Dissolve 0.6602 g of arsenic(III) oxide in 15 ml of 20% sodium hydroxide solution. The solution was transferred into a 500-ml calibrated flask and

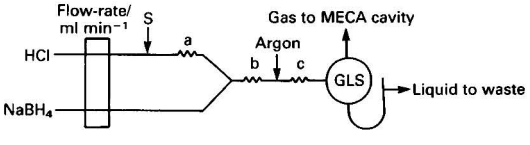


**Fig. 1.** Flow injection manifold for the determination of arsenic with hydride generation and MECA detection. Coils a, b and c, 0.5 mm i.d.; S, point of sample injection; GLS, gas - liquid separator. [NaBH$_4$], 0.2 M; [HCl], 1.5 M; other conditions as in Table 1.

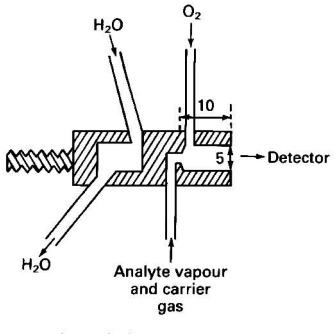


**Fig. 2.** Cross-section of the MECA cavity for FIA - hydride generation system (distances in mm). Conditions as in Table 1

**Table 1.** Experimental parameters used in the FIA - hydride generation - MECA system for the determination of arsenic

| FIA - hydride generation parameters | | MECA parameters | |
|---|---|---|---|
| Sample volume/μl | 120 | Wavelength/nm | 400 |
| Coil b length/cm | 20 | Slit width/nm | 1 |
| Coil c length/cm | 40 | Cavity | At flame centre |
| Solutions pumping rate/ml $min^{-1}$ | 1.5 | Cavity angle below horizontal | 2.0° |
| Carrier gas flow-rate/ml $min^{-1}$ | 0.5 | Flame $H_2$/l $min^{-1}$ | 2.5 |
| HCl concentration in carrier solution/M | 1.5 | Flame $N_2$/l $min^{-1}$ | 4.5 |
| $NaBH_4$ concentration in reductant solution/M | 0.2 | $O_2$ to cavity/ml $min^{-1}$ | 90 |
| EDTA concentration in carrier solution/M | 0.01 | Water cooling flow-rate/ml $min^{-1}$ | 10 |
| NaI concentration in carrier solution/M | 0.2 | | |

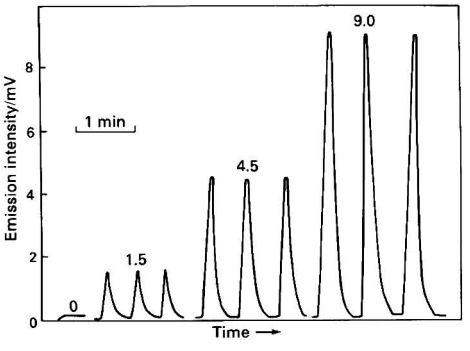


**Fig. 3.** Emission - time responses for arsenic determination (numbers on the peaks indicate the concentration of As in μg $ml^{-1}$). Conditions as in Table 1

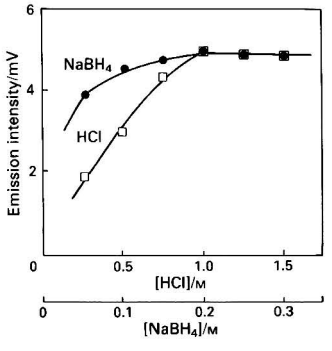


**Fig. 4.** Effect of hydrochloric acid and sodium tetrahydroborate(III) concentrations on the sensitivity taking 5 μg $ml^{-1}$ of As. Conditions as in Table 1

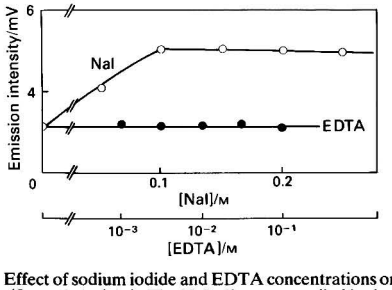


**Fig. 5.** Effect of sodium iodide and EDTA concentrations on arsenic emission (5 μg $ml^{-1}$ of As). The NaI effect was studied in the absence of EDTA and *vice versa*. Conditions as in Table 1

diluted to the mark with 2 M hydrochloric acid. Working solutions were prepared daily by appropriate dilution of the stock solution with 2 M hydrochloric acid.

*Sodium tetrahydroborate(III) solution*, 1 M. Dissolve 3.800 g of powdered laboratory-reagent grade $NaBH_4$ in 100 ml of 0.5 M sodium hydroxide solution. The strong base stabilises the $NaBH_4$ solution.[2]

Preliminary tests and optimisation of the experimental parameters were carried out with a 5 ng $\mu l^{-1}$ As solution.

### Procedure

The instrumental parameters were adjusted to the optimum values (Table 1). The MECA cavity was situated within the flame, and the pump was turned on for a few minutes to fill the U-tube of the gas - liquid separator. The argon used as the carrier gas was allowed to flow. The sample solution was injected via the rotary valve into the flowing stream of $10^{-2}$ M EDTA - 0.2 M sodium iodide - 1.5 M hydrochloric acid, which merged with the 0.2 M $NaBH_4$ flowing stream (Fig. 1). After gas - liquid separation, the generated arsine was carried by the argon carrier gas to the MECA cavity and the transient emission signal from the AsO species[32] was measured at 400 nm against time and recorded. All emission values were based on peak-height measurements.

## Results and Discussion

### Optimisation of Operating Parameters

A systematic investigation was necessary in order to establish the optimum conditions so that a high sensitivity could be achieved with good repeatability. Various chemical and instrumental operating parameters were varied individually while the others were kept constant, and were optimised with respect to the emission intensity. Regarding hydride generation and MECA parameters, the highest peaks with least tailing (Fig. 3) were obtained under the conditions listed in Table 1.

The effects of various chemical parameters are shown in Figs. 4 and 5. In this study, the dependence of analytical sensitivity on the concentration of HCl was examined. The analytical sensitivity increased with increasing HCl concentration from 0.25 to 1.0 M, above which no further increase was observed. The concentration of HCl subsequently used was 1.5 M. It is reported that reduction of arsenate ($As^{5+}$) or arsenite ($As^{3+}$) to $AsH_3$ with $NaBH_4$ is affected by the pH of the solution.[16] $As^{3+}$ is reduced with maximum efficiency in the pH range 0–5, whereas $As^{5+}$ is reduced at pH 0–1. In the present system, the pH was about 0.1 after the merging of the sample and the $NaBH_4$ flowing streams (Fig. 1). Therefore, under the present experimental conditions, total arsenic, including $As^{3+}$ and $As^{5+}$, is measured.

Sodium tetrahydroborate(III) was chosen as a reductant, as it is powerful enough to reduce trivalent and pentavalent arsenic to $AsH_3$.[33] The emission intensity increased with

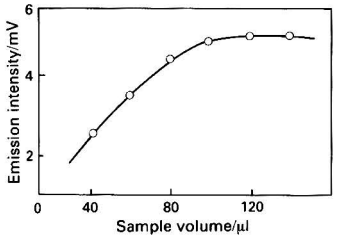


**Fig. 6.** Effect of sample volume on emission intensity for 0.6 μg ml$^{-1}$ of As each time. Conditions as in Table 1

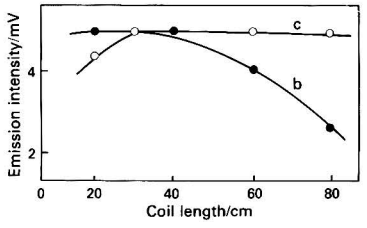


**Fig. 7.** Effect of the lengths of coils b and c on the emission intensity for 5 μg ml$^{-1}$ of As. Conditions as in Table 1

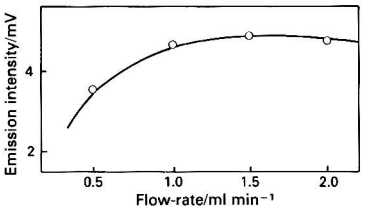


**Fig. 8.** Effect of hydrochloric acid and sodium tetrahydroborate(III) solution pumping rates on the sensitivity for 5 μg ml$^{-1}$ of As. Conditions as in Table 1

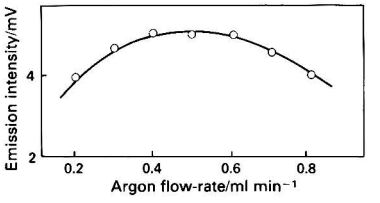


**Fig. 9.** Effect of argon flow-rate on arsenic emission for 5 μg ml$^{-1}$ of As. Conditions as in Table 1

increasing $NaBH_4$ concentration up to 0.2 M (Fig. 4), with no further improvement at higher concentration.

EDTA and sodium iodide were added to the sample carrier solution in order to minimise interferences as described below. The addition of EDTA in the range $10^{-3}$–$10^{-1}$ M did not have any effect on the emission intensity (Fig. 5), whereas the addition of sodium iodide increased the emission intensity at concentrations up to 0.1 M, above which the intensity was independent of concentration. This increase in the emission intensity could be due to the fact that the concentration of iodide effects a complete reduction of the arsenic ions.[27] Concentrations of $10^{-2}$ M EDTA and 0.2 M sodium iodide are recommended.

The influence of sample volume injected is shown in Fig. 6. The sensitivity increased with increasing sample volume, but peak broadening and tailing occurred above 120 μl. A sample volume of 120 μl was found to be reasonable to ensure smooth arsine generation in the FIA system, and therefore a suitable flow of this species into the MECA cavity. In this way the emission from the arsine was restricted within the oxy-cavity.

The effect of different coil lengths was investigated (Fig. 1). In the screening experiments it was established that the length of coil a was unimportant and therefore can be disregarded in the discussion of coil lengths. As illustrated in Fig. 7, the lengths of coils b and c were of great importance. The sensitivity decreased when coil b was longer than 40 cm and increased with increasing length of coil c up to 30 cm. Lengths of coils b and c of 20 and 40 cm, respectively, are recommended.

We used equivalent sample carrier and $NaBH_4$ solution flow-rates. It was found that the emission intensity increased with increasing pumping flow-rates of both solutions (Fig. 8). The emission intensity was found to be almost independent of the pumping flow-rate above 1.0 ml min$^{-1}$. A pumping flow-rate of 1.5 ml min$^{-1}$ in each channel was chosen as the optimum.

The flow-rate of the argon carrier gas was found not to be critical, provided it was held in the range 0.4–0.6 ml min$^{-1}$ (Fig. 9). However, higher flow-rates were found to decrease the emission intensity. This effect could be attributed to an insufficient gas - liquid separation at higher argon flow-rates, and to excessive amounts of gases swept into the cavity (under such conditions, the emission of arsenic occurred in the flame above the cavity, therefore decreasing the sensitivity). When a constant argon flow-rate of *ca.* 0.5 ml min$^{-1}$ was maintained, good sensitivity and reproducibility were obtained.

The MECA parameters, such as position of the cavity, water cooling and flame composition, were also optimised. The cavity was positioned at the centre of the flame, 15 mm above the burner top, and pitched 2° downwards, in line with the detector. The cavity was cooled with water, in order to protect it from incandescence and the support device from over-heating and deformation; the best sensitivity was achieved with a water-cooling rate of 10 ml min$^{-1}$.

It is well known that AsO species are only induced within the cavity by the use of an oxy-cavity.[34] Therefore, an oxygen flow-rate to the cavity (Fig. 2) of 90 ml min$^{-1}$ restricted the emission within the cavity space and made the cavity environment hotter, so improving the sensitivity.

The intensity of the emission of AsO increases with increasing temperature of the cavity.[34] Therefore, the flame composition should be carefully optimised. The optimum temperature for promoting AsO emission was achieved with hydrogen and nitrogen flow-rates of 2.5 and 4.5 l min$^{-1}$, respectively. This flame composition was also found to give the best signal to noise ratio.[35]

### Calibration Graph, Precision and Detection Limit

The calibration graph obtained by injection of 120 μl of arsenic solutions was rectilinear from 0.1 to 10 μg ml$^{-1}$ of As. The maximum emission intensity increased linearly with arsenic concentration as expressed by the equation $E_{As} = -0.12 + 1.52X_{As}$, $r = 0.9997$ (seven points), where $E_{As}$ is the peak height (mV) and $X_{As}$ the arsenic concentration (μg ml$^{-1}$). The relative standard deviations for the determination of 0.8 and 8.0 μg ml$^{-1}$ of As, obtained from eight replicate analyses, were 5.5 and 2.8%, respectively. The detection limit was evaluated by calculating the mean standard deviation of eight sets of replicate blank determinations. The detection limit, considered as twice the signal to blank ratio, was 0.08 μg ml$^{-1}$ (9.6 ng) of As.

**Table 2.** Effect of foreign ions, EDTA and sodium iodide on arsenic emission intensity. Concentrations: arsenic, 1 μg ml$^{-1}$; foreign ions, all at 100 μg ml$^{-1}$; EDTA and NaI in the sample carrier solution, 0.01 and 0.2 M, respectively

| Ion added | Change in emission intensity,* % | | | |
|---|---|---|---|---|
| | As alone | As + NaI | As + EDTA | As + NaI + EDTA |
| $Ni^{2+}$ | −50 | −43 | −3 | −2 |
| $Ag^{+}$† | −25 | −20 | −1 | −1 |
| $Bi^{3+}$ | −30 | −28 | −2 | −1 |
| $Zn^{2+}$ | −10 | −8 | 0 | 0 |
| $Te^{4+}$ | −6 | −5 | −5 | −4 |
| $Se^{4+}$ | −7 | −1 | −7 | −1 |
| $Cu^{2+}$ | −15 | −1 | −10 | −1 |
| $Fe^{3+}$ | −30 | −1 | 0 | 0 |
| $Cr^{3+}$ | −2 | 0 | 0 | 0 |
| $Al^{3+}$ | −1 | 0 | 0 | 0 |
| $Co^{2+}$ | −34 | −33 | −1 | 0 |

* Compared with the emission in the absence of interfering ions.
† AgCl suspension injected.

**Table 3.** Analysis of NBS orchard leaves standard reference material

| Sample No. | Arsenic content/μg g$^{-1}$ | |
|---|---|---|
| | Certified | Measured* |
| 1 | 11 ± 2 | 9.2 ± 0.3 |
| 2 | 14 ± 2 | 12.0 ± 0.6 |
| 3 | 10 ± 2 | 9.5 ± 0.4 |

* Four determinations; 1.0 g of dried orchard leaves sample was taken for each analysis

### Interferences

Comprehensive studies of interferences in the determination of arsenic by hydride generation in several systems have been reported.[36–38] A variety of likely interfering ions were studied in our system under the recommended optimum conditions. An interference was defined as significant if a change of more than two standard deviations in the measurements was observed. Of the ions studied, alkali and alkaline earth elements, $Al^{3+}$ and $Cr^{3+}$ did not interfere, whereas other ions suppressed the emission intensity. Most of these interferences were effectively eliminated or reduced by the addition of EDTA (Table 2), except for $Se^{4+}$ and $Cu^{2+}$. However, the interference effect of these ions was almost eliminated by also including NaI (0.2 M) in the sample carrier solution (Table 2).

### Determination of Arsenic in Standard Samples

In order to evaluate the applicability of the present method to real samples, we determined arsenic in NBS orchard leaves standard reference material. The samples were digested as indicated by Liversage and Van Loon,[27] although the final volume was reduced by evaporation to *ca.* 4–5 ml. The results are in good agreement with the certified values, as shown in Table 3.

## Conclusion

The flow injection - hydride generation system used for the determination of arsenic by MECA is a very simple, sensitive and rapid technique. About 100 measurements per hour can be made. The sensitivity of this method is nearly an order of magnitude less than that reported for atomic absorption spectrometry; however, our method allows the determination of arsenic in real samples, such as plant material, with good accuracy. In addition, the system may also be adaptable for the determination of other hydride-forming species that would generate chemiluminescence emission in a MECA cavity, such as Se, Sb, Sn and Ge, in rocks, sediments and minerals.

## References


1. Haywood, M. G., and Riley, J. P., *Anal. Chim. Acta*, 1976, **85**, 219.
2. Fiorino, J. A., Jones, J. W., and Capar, S. G., *Anal. Chem.*, 1976, **48**, 120.
3. Pierce, F. D., and Brown, H. R., *Anal. Chem.*, 1976, **48**, 693.
4. Cladney, E. S., and Owens, J. W., *Anal. Chem.*, 1976, **48**, 2220.
5. Johnson, D. L., *Environ. Sci. Technol.*, 1971, **51**, 411.
6. Portmann, J. E., and Riley, J. P., *Anal. Chim. Acta*, 1964, **31**, 509.
7. Haywood, M. G., and Riley, J. P., *Anal. Chim. Acta*, 1976, **85**, 219.
8. Reay, P. F., *Anal. Chim. Acta*, 1974, **72**, 145.
9. Thompson, K. C., and Thomerson, D. R., *Analyst*, 1974, **99**, 595.
10. Maruta, T., *Anal. Chim. Acta*, 1975, **77**, 37.
11. Van Loon, J. C., and Brooker, E. J., *Anal. Lett.*, 1974, **7**, 505.
12. Thompson, M., Pahlavanpour, B., Walton, S. J., and Kirkbright, G. F., *Analyst*, 1978, **103**, 568.
13. Pahlavanpour, B., Thompson, M., and Thorne, L., *Analyst*, 1981, **106**, 467.
14. Robbins, W. B., and Caruso, J. A., *Anal. Chem.*, 1979, **51**, 889A.
15. Ebdon, L., Wilkinson, J. R., and Jackson, K. W., *Anal. Chim. Acta*, 1982, **136**, 191.
16. Matsumoto, K., and Fuwa, K., *Anal. Chem.*, 1982, **54**, 2012.
17. Belcher, R., Bogdanski, S. L., Ghonaim, S. A., and Townshend, A., *Anal. Chim. Acta*, 1974, **72**, 183.
18. Belcher, R., Bogdanski, S. L., Ghonaim, S. A., and Townshend, A., *Anal. Lett.*, 1974, **7**, 133.
19. Belcher, R., Bogdanski, S. L., Henden, E., and Townshend, A., *Analyst*, 1975, **100**, 522.
20. Henden, E., *Analyst*, 1982, **107**, 872.
21. Henden, E., Pourreza, N., and Townshend, A., *Prog. Anal. At. Spectrosc.*, 1979, **2**, 355.
22. Tyson, J. F., *Analyst*, 1985, **110**, 419.
23. Lown, J. A., and Johnson, D. C., *Anal. Chim. Acta*, 1980, **116**, 41.
24. Lown, J. A., Koile, R., and Johnson, D. C., *Anal. Chim. Acta*, 1980, **116**, 33.
25. Fogg, A. G., and Bsebsu, N. K., *Analyst*, 1981, **106**, 1288.
26. Astrom, O., *Anal. Chem.*, 1982, **54**, 190.
27. Liversage, R. R., and Van Loon, J. C., *Anal. Chim. Acta*, 1984, **161**, 275.
28. Burguera, J. L., and Burguera, M., *Anal. Chim. Acta*, 1984, **157**, 177.
29. Burguera, J. L., Burguera, M., and Flores, D., *Anal. Chim. Acta*, 1985, **170**, 331.
30. Burguera, M., and Burguera, J. L., *Anal. Chim. Acta*, 1986, in the press.
31. Vijan, P. N., and Wood, G. R., *At. Absorpt. Newsl.*, 1974, **13**, 33.
32. Belcher, R., Bogdanski, S. L., Henden, E., and Townshend, A., *Anal. Chim. Acta*, 1977, **92**, 33.
33. Braman, R. S., Justen, L. L., and Foreback, C. C., *Anal. Chem.*, 1972, **44**, 2195.
34. Burguera, M., Bogdanski, S. L., and Townshed, A., *CRC Crit. Rev. Anal. Chem.*, 1980, **10**, 185.
35. Burguera, J. L., Burguera, M., and Townshend, A., *Acta Cient. Venez.*, 1984, **35**, 165.
36. Haywood, M. G., and Riley, J. P., *Anal. Chim. Acta*, 1976, **85**, 219.
37. Nakahara, T., *Anal. Chim. Acta*, 1981, **131**, 73.
38. Peacock, C. J., and Singh, S. C., *Analyst*, 1981, **106**, 931.


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# Determination of Sulphur, Phosphorus, Magnesium, Silicon and Aluminium in Washing Powders by X-Ray Fluorescence Spectrometry

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A method for preparing and analysing samples of washing powders by X-ray fluorescence spectrometry is described. The sample preparation involves the use of an automatic machine for the preparation of fused dilithium tetraborate beads and demonstrates the feasibility of using this technique for difficult samples containing a mixture of organic and inorganic components. The results of the X-ray fluorescence method are compared with those from wet chemical analysis. The determination of total sulphur, phosphorus, silicon, magnesium and aluminium by X-ray fluorescence represents a considerable time saving compared with wet chemical analysis.

**Keywords:** *Washing powders; X-ray fluorescence spectrometry; sulphur, phosphorus, silicon, magnesium and aluminium determination; automatic sample preparation*

A wide range of compounds are used in the formulation of washing powders; in addition to organic surfactants, optical brighteners and softening agents, a washing powder may contain phosphates, sulphates, silicates, magnesium salts of bleaching agents, etc. The analysis of washing powders for elements such as sulphur, phosphorus, silicon, aluminium and magnesium can be a time-consuming operation and normally involves the use of several different analytical techniques.

A widely used method of analysis involves initial fusion with a mixed nitrate - carbonate flux, to destroy organic matter, followed by dissolution of the fused melt and determination of individual elements by a combination of gravimetric, spectrophotometric and physical methods.[1]

X-ray fluorescence spectrometry is a technique that can be used to determine a number of elements quickly on one sample, and usually the sample for analysis is prepared as a fused dilithium tetraborate bead. The initial fusion of the sample therefore is common to both the traditional and X-ray techniques of analysis, but the preparation of a bead followed by X-ray fluorescence analysis should save time and effort compared with traditional techniques.

In the preparation of a sample bead for X-ray fluorescence analysis, the direct fusion of the sample with a flux such as dilithium tetraborate is perfectly satisfactory for inorganic materials in a fully oxidised state. However, problems are encountered if the sample contains organic matter or metals in a reduced form. In these instances the sample has to be fully oxidised before a fused bead can be made. Washing powders require such an oxidation step before fusion.

This paper describes a method whereby the total concentrations of the above elements can be rapidly determined by an X-ray fluorescence technique using a sample in the form of a single fused dilithium tetraborate bead. The manual effort required for sample preparation is kept to a minimum by using an automatic bead maker.

## Experimental

### Apparatus and Reagents

A Perl 'X-2 automatic bead machine, manufactured by Soled and supplied by Laborlux S.A., Luxembourg, was used for sequential oxidation and fusion of the sample into a dilithium tetraborate bead. This is a programmable radiofrequency furnace unit, which can also agitate the molten sample and pour the melt into a casting mould.

The crucible for sample fusion and the casting mould were made from a platinum - gold alloy. The mould produced beads of 25 mm diameter.

A Philips PW1400 X-ray fluorescence spectrometer with a Philips P851M minicomputer was used for the fluorescence measurements.

The dilithium tetraborate used for the sample fusion was Spectroflux 100 from Johnson Matthey Chemicals Ltd. Other reagents used were of analytical-reagent grade.

These operations produce a melt that is sufficiently fluid to give a flat area on top of the bead suitable for X-ray fluorescence measurement.[2] The beads must be handled with clean gloves to avoid contamination and to prevent cuts from sharp edges.

### Calibration

A series of standard calibration beads were prepared from mixtures of sodium sulphate, sodium orthophosphate, silicon dioxide, aluminium oxide and magnesium oxide. The ranges covered were equivalent to samples containing 0–250 g $kg^{-1}$ of $SO_4$, 0–400 g $kg^{-1}$ of $SiO_2$ and $PO_4$, 0–200 g $kg^{-1}$ of $Al_2O_3$ and 0–100 g $kg^{-1}$ of MgO. A reference bead was prepared, containing all five elements at about the sample equivalent of the 50 g $kg^{-1}$ level, which was placed in the monitor position of the X-ray spectrometer.

**Table 1.** X-ray spectrometer operating parameters

| Parameter | S | P | Si | Mg | Al |
|---|---|---|---|---|---|
| Peak angle/°2θ | 110.710 | 141.030 | 144.710 | 45.165 | 145.125 |
| Background angle/°2θ | 111.710 | 140.030 | 143.710 | 44.165 | 146.625 |
| Crystal | Germanium | Germanium | Indium antimonide | Thallium acid phthalate | Pentaerythritol |

X-ray fluorescence measurements were made using the conditions given in Table 1 for each element in each calibration sample. The X-ray count for each measurement was corrected for background and the ratio of corrected count against the corrected monitor count, for the same element, was calculated. The use of a count ratioed to a monitor bead compensates for any long-term instrumental drifts.

A regression analysis was made to calculate the inter-element interferences and to obtain a calibration equation for each element. These calculations were made using a Philips 851 computer operating with Philips X14 An2-3 (UK) software.

The operating parameters of the X-ray spectrometer are shown in Table 1. A rhodium tube operating at 50 kV and 50 mA was used for all measurements. All measurements were of 50 s duration, with a coarse collimator and a flow proportional counter. The peak angles given were for the Kα peak of the element in each instance.

### Sampling

Washing powders are generally heterogeneous in nature, so care must be taken in sampling from packets. The procedure used in our laboratories was to rotary divide successively the contents of an entire packet down to a mass of approximately 30 g. This sample was then ground to a fine powder in a high-speed blade mill to provide a homogeneous, representative sample from the packet, for subsequent analysis.

### Preparation of Samples for X-ray Fluorescence Analysis

The sample fusion used was a two-stage procedure.[3-5] In the first, the oxidation stage, 0.25 g of the ground sample was mixed with 1 g of sodium carbonate and 1 g of a mixture containing 3 parts by mass of sodium nitrate and 1 part by mass each of potassium nitrate and strontium nitrate. This mixture was heated in a platinum - gold crucible at approximately 800 °C for 6 min without any agitation. In the second, the fusion stage, this mixture was agitated with 5 g of dilithium tetraborate at approximately 950 °C for 3 min. All weighings were carried out to ±0.001 g. The fused melt was finally poured into a casting mould to give a glass bead suitable for X-ray fluorescence analysis.

The oxidation and fusion stages were carried out using the automatic bead machine. The microprocessor in this instrument controls six parameters, namely time, heating power, crucible movement, angle through which the crucible is rotated, the speed of movement and the presence or absence of cooling air. The microprocessor also allows these parameters to be grouped into up to six operations for the preparation of the bead.

In the sequence for this particular fusion, the sample and the nitrate - carbonate mixture were placed on one side of the crucible and the dilithium tetraborate on the other. The first heating period, without any agitation, allows complete oxidation of any organics in the sample. The second heating period, with agitation, then mixes the oxidised sample with the tetraborate and produces a homogeneous melt.

A similar fusion technique can be carried out manually. In this instance, the oxidation stage is carried out in a furnace at 800 °C for 6–10 min. The fusion stage is then carried out either in a second furnace at 950 °C, or over the flame of a Meker burner. The fusion time for the second stage for manual operation is about 20 min, with manual swirling at 5-min intervals. The melt is poured into a heated casting mould to give a glass bead similar to that prepared on the automatic bead machine.

**Table 2.** X-ray calibration data

| Element | Concentration range/g kg$^{-1}$ | σ/g kg$^{-1}$ | $K$ |
|---|---|---|---|
| S as $SO_4$ | 0–250 | 4.0 | 1.4 |
| P as $PO_4$ | 0–400 | 1.7 | 0.6 |
| Si as $SiO_2$ | 0–400 | 4.9 | 1.3 |
| Mg as MgO | 0–100 | 3.0 | 1.6 |
| Al as $Al_2O_3$ | 0–200 | 2.5 | 2.2 |

## Results

### Calibration

The calibration equations for each element were calculated using the Philips X14 software, using the de Jongh model, with influence coefficients (α) calculated from the experimental data during the regression analysis.

The values of the standard deviation, σ, and the proportionality factor, $K$, from the regression analyses are given in Table 2.

### Precision

The precision of the sample preparation and measurement was demonstrated by fusing and measuring six sample beads from one ground sample. The results are shown in Table 3.

With the exception of magnesium, the relative standard deviation of 1–3% for the sample preparation and measurement was of the same order as the over-all accuracy that could be expected from residual sample mass fluctuations on differing samples. The precision of the method for these four elements therefore satisfied our original aims. The poorer precision for magnesium, with a relative standard deviation of 10%, reflects the low concentration being measured and the fact that magnesium is the lightest of these elements.

### Accuracy

The samples, which contain a mixture of organic and inorganic components, were prepared by oxidation and fusion with fixed masses of flux. The loss of volatile material during fusion is the limiting factor in the accuracy of the method, which is about ±2% relative.

The accuracy of the method was demonstrated by analysing a known washing powder base and then adding known

**Table 3.** Precision of sample preparation and X-ray measurement

| | Concentration/g kg$^{-1}$ | | | | |
|---|---|---|---|---|---|
| Fusion No. | Mg | $SO_4$ | $PO_4$ | $SiO_2$ | $Al_2O_3$ |
| 1 | 27 | 107 | 203 | 39 | 48 |
| 2 | 28 | 107 | 204 | 39 | 49 |
| 3 | 26 | 106 | 203 | 39 | 48 |
| 4 | 27 | 107 | 208 | 39 | 48 |
| 5 | 30 | 110 | 207 | 39 | 48 |
| 6 | 35 | 106 | 203 | 37 | 47 |
| Standard deviation | 3.3 | 1.5 | 2.2 | 0.85 | 0.63 |
| Relative standard deviation, % | 11.4 | 1.4 | 1.1 | 2.2 | 1.3 |

**Table 4.** Analysis of washing powders of known composition

| | | Concentration/g kg$^{-1}$ | | | | |
|---|---|---|---|---|---|---|
| Sample No. | | Mg | $SO_4$ | $PO_4$ | $SiO_2$ | $Al_2O_3$ |
| A | Known | 0 | 178 | 339 | 66 | 0 |
| | X-ray | 2 | 182 | 342 | 69 | <1 |
| B | Known | 14 | 142 | 271 | 53 | 25 |
| | X-ray | 13 | 135 | 272 | 49 | 23 |
| C | Known | 28 | 107 | 203 | 40 | 50 |
| | X-ray | 29 | 107 | 205 | 39 | 48 |
| D | Known | 42 | 71 | 136 | 26 | 75 |
| | X-ray | 40 | 67 | 135 | 26 | 78 |

**Table 5.** Comparison of wet chemical and X-ray results

| | | Concentration/g kg$^{-1}$ | | | | |
|---|---|---|---|---|---|---|
| Sample No. | Method | Mg | $SO_4$ | $PO_4$ | $SiO_2$ | $Al_2O_3$ |
| 1 | Chemical | — | 188 | 1 | 42 | — |
| | X-ray | <0.1 | 191 | 1 | 36 | <0.1 |
| 2 | Chemical | — | 191 | 295 | 53 | — |
| | X-ray | <0.1 | 196 | 289 | 51 | <0.1 |
| 3 | Chemical | — | 151 | 313 | 37 | — |
| | X-ray | <0.1 | 149 | 314 | 39 | <0.1 |
| 4 | Chemical | — | 153 | 325 | 63 | — |
| | X-ray | <0.1 | 150 | 330 | 59 | <0.1 |

amounts of magnesium and aluminium to the base. The magnesium was added as magnesium monoperoxyphthalate and the aluminium as aluminium oxide. In each instance 30-g aliquots of material were prepared and ground in a high-speed grinder with stainless-steel blades to give a homogeneous sample. The results are given in Table 4.

These results are in agreement within about ±2%, which is the limit of the accuracy expected. As a further check on the accuracy, a number of samples of commercial washing powders were analysed by X-ray fluorescence and by wet chemical methods for sulphur, silicon and phosphorus.

The wet chemical analysis was carried out on the solution resulting from dissolution of the melt from a sodium carbonate - sodium nitrate fusion. Sulphate was determined gravimetrically as barium sulphate, silicon by acid precipitation followed by volatilisation with hydrofluoric acid and phosphate spectrophotometrically with molybdovanadate. The results are given in Table 5. These results again are within the expected ±2%.

## Discussion

The total analysis of washing powders is a complex procedure. The X-ray method described here gives a determination of total sulphur, phosphorus, silicon, aluminium and magnesium. Sulphur particularly can exist in inorganic forms such as sodium sulphate and organic forms such as anionic surface-active agents. If necessary, these different forms of sulphur can be characterised by carrying out a preliminary separation of organic and inorganic fractions by solvent extraction, chromatography, etc. Alternatively, if the sulphur is present as an anionic surface-active agent then this component can be titrated with a cationic titrant.[1]

Similarly, silicon can be present as both sodium silicate and as zeolite. In this instance the aluminium and silicon figures would have to be used together to assess the formulation.

## Conclusions

X-ray fluorescence spectrometry combined with an automated bead maker provides a rapid method for the determination of total sulphur, phosphorus, silicon, aluminium and magnesium in washing powders. The results obtained are within ±2% relative of those obtained by wet chemical analysis.

The oxidative fusion technique described demonstrates the feasibility of preparing glass beads for X-ray fluorescence spectrometry from difficult samples containing varying amounts of organic and inorganic constituents.

## References

1. Longman, G. F., "The Analysis of Detergents and Detergent Products," Wiley, Chichester, 1975.
2. Oliver, G. J., *Eur. Spectrosc. News.*, 1976, No. 8, 4.
3. Petin, J., Bentz, F., and Wagner, A., paper presented at the International Conference on Industrial Inorganic Elemental Analysis, Metz, June 1st–5th, 1981.
4. Wagner, A., Petin, J., Hein, J., and Bentz, F., "Spectrometertagung," Walter de Gruyter, Berlin, New York, 1981, pp. 71–81.
5. Petin, J., and Wagner, A., *Labor Praxis*, 1983, May, 416.

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# Determination of α-Impurities in the β-Polymorph of Inosine Using Infrared Spectroscopy and X-ray Powder Diffraction

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An X-ray powder diffraction method for the quantitative determination of the α-inosine content of mixtures of the crystalline polymorphic forms of α- and β-inosine is described. Oriented sample discs are prepared by pressing with a cellulose binder and the α-inosine content is calculated from measurements of the 8.25 Å and 7.25 Å X-ray diffraction intensities of α- and β-inosine, respectively. The method has a relative deviation of 7% and a detection limit of 0.4% α-inosine. This represents a 35-fold improvement in sensitivity over conventional infrared spectroscopy and a 14-fold improvement over Fourier transform infrared spectroscopy.

**Keywords:** *α-Inosine determination; polymorphs; X-ray diffraction; infrared spectroscopy; pharmaceuticals*

There is a growing interest in the pharmaceutical applications of polymorphism[1] and hence in techniques for its identification and quantification. It is also required routinely by some national drug regulatory agencies that specific methods for detecting the absence of unwanted polymorphs be developed as routine quality control assays. Such unwanted polymorphs will not necessarily have any intrinsic toxicity but they must nevertheless be quantified.

Inosine, $C_{10}H_{12}N_4O_5$, crystallises in two polymorphic anhydrous forms, one of which is orthorhombic and one monoclinic.[2,3] Inosine also forms a dihydrate that is monoclinic. Suzuki and Nagashima[2] designated the orthorhombic form of anhydrous inosine the α-form and the monoclinic form of anhydrous inosine the β-form. In our work it was important to detect and quantify, with a detection limit of less than 5%, the presence of α-inosine in samples of the β-polymorphic form. Preliminary investigations were made using differential-scanning calorimetry (DSC) but this technique proved unsuitable because of the closeness of the melting-points of the two polymorphic forms. We therefore investigated the suitability of infrared spectroscopy and X-ray powder diffraction methods for this purpose.

The application of infrared spectroscopy to the analysis of pharmaceutical compounds is well known. However, no methods for the determination of inosine in the solid state appear to have been reported. Quantitative X-ray diffractometry is a technique that has considerable potential in the assay of pharmaceutical materials and was used by Christ *et al.*[4] in their determination of crystalline sodium penicillin G. Shell[5] pointed out that "published reports on quantitative diffraction applications to organic systems are almost non-existent," and described several methods of determining crystalline components in drug systems.

However X-ray powder diffraction has continued to find only occasional pharmaceutical application, such as in the analysis of intact tablets by Papariello *et al.*[6] and oral suspensions by Kuroda.[7] More recently Imaizumi *et al.*[8] reported a determination of the degree of crystallinity of indomethacin using lithium fluoride as an internal standard. A useful review is given by Zwell and Danko.[9] An X-ray diffraction analysis of mixtures of inosine polymorphs was described briefly by Suzuki[10] and this formed the basis of our method.

## Experimental

### Instrumentation

Conventional infrared spectra were obtained using a Pye Unicam SPS 300 instrument. Spectra were also recorded using a Nicolet 5MX Fourier transform infrared spectrometer using a continuous scan Michelson Interferometer, S/5 optics with spectral range 4600–400 $cm^{-1}$, laser-assisted sample alignment with a resolution of 4 $cm^{-1}$ and a wavelength accuracy of 0.01 $cm^{-1}$ and automatic gain. A standard glower source was used and a two-pen digital plotter with variable abscissa and ordinate expansion.

X-ray diffraction measurements were made using a Philips PW 1050 wide-range goniometer with a 1° dispersion slit, a 0.2-mm receiving slit and a 1° anti-scatter slit. The Cu anode X-ray tube was operated at 40 kV and 20 mA in combination with a Ni filter to give monochromatic Cu Kα X-rays of wavelength 1.5418 Å. The angular calibration of the goniometer was based on the (020) reflection of cholesterol[11] at 5.260 °2θ.

### Materials

*α-Inosine, crystalline.* Ajinomoto Co., Inc., Japan.
*β-Inosine, crystalline.* Ajinomoto Co., Inc., Japan.
*Cellulose powder, Whatman CF* 11.

### Sample Preparation

For the infrared spectroscopic studies potassium bromide discs containing 1% of inosine were prepared by compression under vacuum at a pressure of 10 ton.

To prepare samples for X-ray diffraction analysis the following method is used. Grind together by hand 1.00 g of inosine sample and 1.00 g of cellulose powder using an agate pestle and mortar. Transfer the mixture into a glass vial, add two 6 mm diameter polystyrene balls, stopper and further homogenise the mixture by shaking for 5 min in a Glen Creston ball mill. Transfer the resulting powder into a 32 mm diameter stainless-steel die and press into a disc at a pressure of 10 ton. The reasons for adopting this method of sample

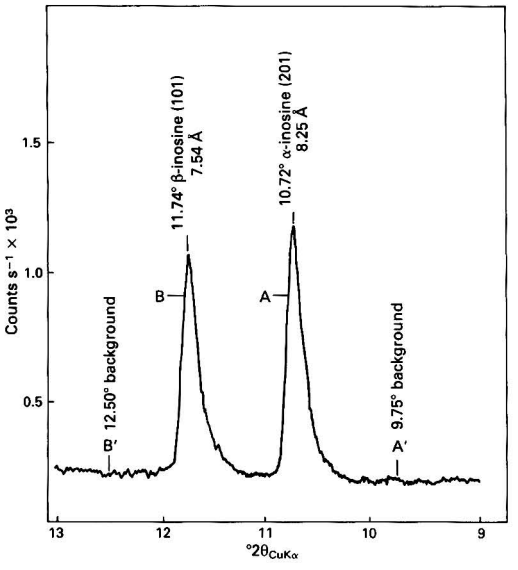


**Fig. 1.** Portion of X-ray diffraction chart of a mixture of α-inosine (30%) and β-inosine (70%) showing the measurements used to derive the intensity values $I_\alpha$ and $I_B$. Pressed disc, cellulose binder. $I_\alpha = A - A'$; $I_B = B - B'$. Indexing of peaks from Suzuki and Nagashima[2]

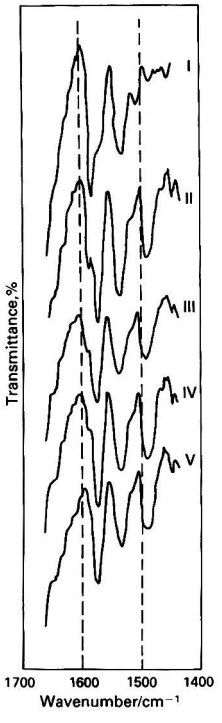


**Fig. 2.** Infrared transmittance spectra of inosine mixtures. I, 50%; II, 25%; III, 20%; IV, 15%; and V, 10% α-inosine

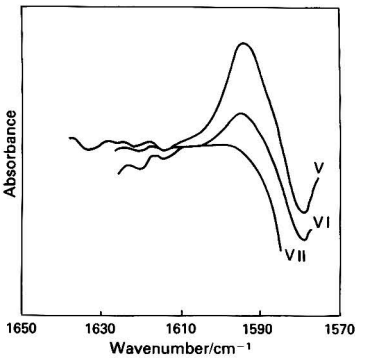


**Fig. 3.** Absorbance spectra of inosine mixtures acquired using the Fourier transform IR spectrometer. V, 10%; VI; 5% and VII, 2.5% α-inosine

preparation as opposed to the conventional cavity mounts are discussed in detail below.

### Procedure for X-Ray Diffraction Analysis

Insert the inosine - cellulose disc into the diffractometer and record the number of counts obtained in 60 s at 9.75 °2θ (α-inosine background), 10.72 °2θ (α-inosine peak), 11.74 °2θ (β-inosine peak) and 12.50 °2θ (β-inosine background). Subtract the appropriate background counts from the peak counts to give the diffracted intensity values $I_\alpha$ and $I_\beta$ for α- and β-inosine, respectively (Fig. 1). Calculate the α-inosine concentration, $C_\alpha$ from the following equation:

$$C_\alpha = \frac{100 I_\alpha}{(I_\alpha + 2.52 I_\beta)} \%$$

Determine also the blank using a sample of pure β-inosine reference standard.

It is assumed that a preliminary diffractogram of the sample will have been recorded in order to check the peak positions and to verify that interfering peaks are absent. All our samples were known to contain only anhydrous inosine.

## Results and Discussion

### Infrared Methods

Conventional infrared spectra provided a rapid means of identifying the form of inosine present. Visual examination of the spectra suggested that the α-band at 1593 $cm^{-1}$ and the β-band at 1577 $cm^{-1}$ were the most suitable bands for quantification by the base-line method.[12] Transmittance spectra illustrating these bands for inosine mixtures containing 10, 15, 20, 25 and 50% of the α-polymorph are shown in Fig. 2. The peak at 1593 $cm^{-1}$ is visible in spectra of samples containing 15% or greater of the α-form, but was absent in samples containing 10% or less of α-inosine. The detection limit for α-inosine was improved by using the Fourier transform instrument. Absorbance spectra (with subtraction) of inosine mixtures containing 10, 5 and 2.5% α-inosine are shown in Fig. 3 and indicate that α-inosine may be detected down to about 5% by this method.

### X-Ray Diffraction Method

*Peak height* versus *peak area*

On many diffractometers it is easier to measure peak heights rather than peak area and for the sake of simplicity we have adopted this practice here. In some instances peak-height measurement also has the added advantage of avoiding interference from adjacent peaks. On the other hand,

peak-area measurement can result in improved precision and compensates for the loss of intensity owing to crystal defects and small particle size.

*Preferred orientation effects*

In our preliminary investigation of an X-ray diffraction assay for inosine polymorphs we used conventional cavity mounts. However, satisfactory precision could not be obtained and this was attributed to the crystal morphology of the two phases. α-Inosine forms long needle-like crystals while β-inosine crystals have a platy habit (shape), and it is very difficult to make a randomly oriented cavity mount of either of these materials because of the tendency for the crystals to adopt preferred orientations. Consequently, the reproducibility of X-ray diffraction intensities from cavity mounts is very poor even when careful packing procedures are employed. It is worth pointing out that preferred orientation affects (enhances or diminishes) all X-ray reflections from a crystalline powder and not just those arising from lattice planes parallel to the orientation. The problem cannot, therefore, be approached by choosing to measure a different reflection. An attempt was made to overcome these orientation effects by grinding the sample with a small amount (2%) of carbon black, as recommended for this purpose by Christ *et al.*[4] However, no improvement in precision could be obtained by this means and scanning-electron micrographs of the resulting powders showed the characteristic morphology of the inosine crystals to be unchanged. The use of cavity mounts was then abandoned and instead *highly oriented* mounts were made in the form of pressed discs with a cellulose binder. These discs are self supporting, easy to handle and can be inserted directly into the diffractometer. A comparison of the diffraction patterns of both α-inosine and β-inosine showed little difference in relative peak intensities between the cavity mounts and the pressed discs, confirming that a high degree of preferred orientation was already present in the cavity mounts. The reproducibility of X-ray intensities from the pressed discs was, however, greatly improved and this method of sample preparation was therefore adopted. No deleterious effects on crystal structure owing to pressure were observed.

It is possible that different crystallisation regimes may give rise to crystals of different habit and hence different orientation behaviour. The grinding step is therefore of considerable importance as grinding alters the original crystal habit by producing cleavage flakes, thus ensuring that the morphology of the grains in the resulting powder is uniform for any given phase. It also ensures that the habits present in both standard and sample are essentially the same.

*Accuracy*

The accuracy of the X-ray method was assessed by analysing inosine mixtures containing 0, 0.5, 1, 10, 20, 30, 40 and 50% of the α-polymorph. The results are shown in Table 1. The relationship between the α-inosine concentration taken and the α-inosine concentration found is described by a linear regression equation with slope equal to 0.997 and an intercept of 0.20% of α-inosine. This closely approximates to the ideal line of unit slope and zero intercept.

**Table 1.** Results of X-ray diffraction analysis of mixtures containing known masses of inosine polymorphs

| Amount of α-inosine, % | | | |
|---|---|---|---|
| Taken | Found* | $n$ | $\sigma_{n-1}$ |
| 0.00 | 0.002 | 8 | 0.10 |
| 0.50 | 0.54 | 8 | 0.14 |
| 1.00 | 0.90 | 8 | 0.13 |
| 10.00 | 11.45 | 2 | — |
| 20.00 | 19.31 | 8 | 1.47 |
| 30.00 | 30.24 | 2 | — |
| 40.00 | 40.49 | 2 | — |
| 50.00 | 49.72 | 2 | — |

* Mean of $n$ replicate analyses of the same disc.

*Precision*

The precision of the method was determined from the analysis of eight replicate discs made from an approximately 1 + 3 mixture of α-inosine and β-inosine by mass. The mean α-inosine concentration found was 24.18% *m/m* with a standard deviation of 1.68% *m/m*. This represents a relative deviation of 6.95%. Counting statistics alone account for a relative deviation of about 2%.

*Detection limit*

In this work it was anticipated that most of the inosine samples that would be assayed would not contain any detectable amount of α-inosine. An accurate determination of the detection limit of the method was therefore of paramount importance. Accordingly, two approaches were adopted for the estimation of the detection limit. The first approach was based on the standard deviation of the blank. A blank disc was prepared using a pure β-inosine reference standard. This standard was considered to be pure because no α-inosine peak was present in the X-ray diffraction pattern and measurement of the parameter $I_\alpha$ gave a value identical to that of pure cellulose. Eight replicate analyses of this disc had a standard deviation of 0.095% *m/m*. The detection limit can therefore be taken to be three times this value, *i.e.*, 0.29% α-inosine. However, we consider this to be an over-optimistic estimate of the detection limit because it assumes that the noise characteristics of the background are similar to the noise characteristics of the signal.

In X-ray diffraction methods this is not a valid assumption as a significant component of the signal noise is likely to be represented by non-reproducibility in the sample preparation technique. The second approach is an extension of the first and is more realistic in that it incorporates information about the analytical precision into the definition of detection limit. Following the theoretical model of the relationship between precision and concentration developed by Thompson and Howarth[13] the detection limit is defined as the concentration at which the precision is 100%. For a 3σ detection limit this value can be found from the following equation:

$$C_d = \frac{300\sigma_0}{(100 - 3k)}$$

where $C_d$ is the detection limit, $\sigma_0$ is the standard deviation at zero concentration and $k$ is the precision of the method at high concentrations expressed as a relative deviation. Substituting the values $\sigma_0 = 0.095$ and $k = 6.95$ leads to a detection limit of 0.4% α-inosine.

*Calculation of α-Inosine Concentration from $I_\alpha$ and $I_\beta$ Values*

The equation already given for the calculation of α-inosine concentrations can be derived from the fundamental equation for quantitative X-ray diffraction stated by Klug and Alexander.[14] The diffracted X-ray intensity ($I$) arising from a component of a mixture is given by the equation

$$I = \frac{kx}{\rho[x(\mu - \mu_m) + \mu_m]} \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where $k$ is a constant depending on the nature of the component, $x$ the mass fraction of the component, $\rho$ the density, $\mu$ the mass absorption coefficient and $\mu_m$ the mass absorption coefficient of the matrix. In the special case of

mixtures of polymorphic forms of a substance, as is the case with the inosine system, we have

$$\mu = \mu_m$$

and therefore equation (1) can be simplified to

$$I = \left(\frac{k}{\rho\mu}\right) x \text{ or } I = Kx$$

where the intensity is directly proportional to the concentration and the constant $K$ is a measure of the sensitivity.

For a binary mixture of two components, A and B, we obtain the two equations:

$$I_A = K_A x_A \quad .. \quad .. \quad .. \quad (2)$$

and

$$I_B = K_B x_B \quad .. \quad .. \quad .. \quad .. \quad (3)$$

As A and B are the only components (the cellulose binder can be neglected),

$$x_B = 1 - x_A$$

Substituting this value of $x_B$ in equation (3) and combining equations (2) and (3) leads to the equation

$$x_A = \frac{I_A}{I_A + [(K_A/K_B) I_B]} \quad .. \quad .. \quad (4)$$

The ratio $K_A/K_B$ is the ratio of the sensitivities of the two components A and B.

The value of this ratio was determined by measuring the diffraction intensities of the α- and β-diffraction peaks using a disc pressed from a mixture of equal masses of the two components and was found to be 2.52. It would be advisable for other workers to determine their own values for this ratio as it may vary slightly between different instruments and different laboratory procedures.

Interestingly, Chung[15] has shown that relationships similar to equation (4) are applicable to any binary system and not only those consisting of two polymorphs. This means that the method of calculation described above can be used in a wide range of pharmaceutical assays.

## Conclusion

X-ray powder diffractometry, in combination with a pressed-disc sample-mounting technique, provides an accurate and sensitive method for determining the polymorphic composition of crystalline inosine samples. The sensitivity is 35-fold greater than that obtainable using conventional infrared spectroscopy and 14-fold greater than that using Fourier transform infrared spectroscopy. X-ray diffraction methods such as this are capable of application to a wide variety of pharmaceutical assays.

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## References

1. Haleblian, J., and McCrone, W., *J. Pharm. Sci.*, 1969, **58**, 911.
2. Suzuki, Y., and Nagashima, N., *Bull. Chem. Soc. Jpn.*, 1970, **43**, 1600.
3. Munns, A. R. I., and Tollin, P., *Acta Crystallogr.*, 1970, **B26**, 1101.
4. Christ, C. L., Barnes, R. B., and Williams, E. F., *Anal. Chem.*, 1948, **20**, 789.
5. Shell, J. W., *J. Pharm. Sci.*, 1963, **52**, 24.
6. Papariello, G. J., Letterman, H., and Huettemann, R. E., *J. Pharm. Sci.*, 1964, **53**, 663.
7. Kuroda, K., *J. Pharm. Sci.*, 1968, **57**, 250.
8. Imaizumi, H., Nambu, N., and Nagai, T., *Chem. Pharm. Bull.*, 1980, **28**, 2565.
9. Zwell, L., and Danko, A. W., *Appl. Spectrosc. Rev.*, 1975, **9**, 167.
10. Suzuki, Y., *Bull. Chem. Soc., Jpn.*, 1974, **47**, 2549.
11. Kittrick, J. A., *Proc. Soil Sci. Soc. Am.*, 1960, **24**, 17.
12. Williams, W. D., *in* Beckett, A. H., and Stenlake, J. B., *Editors,* "Practical Pharmaceutical Chemistry," Part 2, Third Edition, Athlone Press, London, 1976, p. 331.
13. Thompson, M., and Howarth, R. J., *Analyst*, 1976, **101**, 690.
14. Klug, H. P., and Alexander, L. E., "X-ray Diffraction Procedures for Polycrystalline and Amorphous Materials," Second Edition, Wiley-Interscience, New York, 1974.
15. Chung, F. H., *J. Appl. Crystallogr.*, 1974, **7**, 519.

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# Continuous Spectrophotometric Monitoring of Chlorine in Air

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Spectrophotometric monitoring of atmospheric free chlorine, based on the reaction with 4-nitroaniline, was assessed. A Beckman air quality Acralyzer Model K-1008 for nitrogen dioxide monitoring was modified and used for this purpose. Some reducing and oxidising compounds interfered with chlorine measurements, but these were removed efficiently using a scrubber consisting of chromic acid (prepared from phosphoric acid and chromium trioxide) absorbed in silica gel. This scrubber was found to be suitable for chlorine measurements in the presence of hydrogen chloride. Humid conditions resulted in some absorption of chlorine in the scrubber. At 69 °C no such absorption was observed. Ozone was removed either by a glass-beads scrubber heated to 60–65 °C or by the addition of sodium nitrite to the absorbing solution. The sensitivity achieved using this monitor was 0.25 p.p.m. full-scale with solution and air flow-rates of 1 ml $min^{-1}$ and 2 l $min^{-1}$, respectively.

**Keywords:** *Chlorine monitoring; spectrophotometric monitoring; air monitoring*

Various continuous chlorine monitors, based on different theoretical principles, have been developed and manufactured commercially by many companies. Some monitoring instruments have been in use within industrial areas for measuring high levels of chlorine, such as the personal monitors described by Langhorst.[1] Others, used for ambient air monitoring, need to be more sensitive and selective owing to the greater interference of other pollutants.

Some of the methods used for ambient chlorine monitoring include spectrophotometry, electrochemistry and indirect chemiluminescence. All of these suffer from interferences from other pollutants either oxidising or reducing. The extent of the interference differs from one to another depending on the method and on the way in which the monitor was manufactured.

A spectrophotometric method, based on the reaction of chlorine with 4-nitroaniline in the presence of a barbitone sodium catalyst, was developed in our institute.[2] This method exhibited some advantages over other methods using wet chemistry.[2] The aim of this study was to assess this method for the continuous monitoring of chlorine in air.

Two types of scrubbers are needed for the removal of interfering compounds: (1) those for reducing compounds and (2) those for oxidising compounds.

1. A large negative interference is caused by reducing sulphur compounds[2] such as sulphur dioxide, hydrogen sulphide and thiols. In order to remove these interferences, it was necessary to use oxidising scrubbers, such as those used for monitoring oxidants. These scrubbers consisted of chromic acid absorbed on various inert carriers such as a fibre-glass filter,[3] silica gel,[3,4] pumice, boric acid[4] and quartz chips.[4,5] Chromic acid was prepared by mixing chromium trioxide and sulphuric[3] or phosphoric[4,5] acid.

The scrubber, consisting of chromium trioxide, sulphuric acid and a fibre-glass filter, was originally prepared to remove sulphur dioxide.[3] This scrubber was found to be efficient also in removing other reducing sulphur compounds, in addition to ammonia and hydrogen peroxide.[2] No significant oxidation of HCl[2] or NaCl[3] was observed using this scrubber.

This scrubber was tried in the monitoring of chlorine at the Electrochemical Industries Frutarom factory, which is located along the seashore in the city of Acre, Israel, which has a very humid climate. The scrubber used became wet within a few days under the usual working conditions and it was therefore necessary to develop scrubbers having a higher capacity and higher resistance to humidity.

It was thought that scrubbers made of silica gel and chromic acid would have higher oxidation capacities and for this reason the oxidation of chlorides by these scrubbers was studied.

2. Negative interference is caused also by ozone. Wartburg *et al.*[6] eliminated this interference by passing the sampled air over heated scrubbers consisting of stainless-steel frits. Lindqvist[4] used heated silver, aluminium or platinum scrubbers for this purpose. In this work heated glass beads were tried. The effect on ozone removal of adding sodium nitrite to the reacting solution was also investigated.

## Experimental

All chemicals were of analytical-reagent grade, unless indicated otherwise.

### Chemical Method

The spectrophotometric method used was based on the chemical reaction of free chlorine with an alkaline solution of 4-nitroaniline in the presence of a barbitone sodium catalyst.[2] The change in the absorbance of the solution at 485 nm is linearly proportional to the concentration of chlorine.

### Analyser

A Beckman air quality Acralyzer Model K-1008 for continuous monitoring of nitrogen dioxide was used. The instrument consists of three units: an analyser, a controller and a recorder. The analyser is made up of a visible-range ratio photometer, a solution metering pump, an air pump, a glass flow system and a flow meter. The following modifications were made.

1. The original optical filter was replaced with a Corning No. 4060 CS-4-67 1.88-mm optical filter, suitable for reading the absorbance of the absorbing solution.

2. The gain and the ratios of the Wheatstone bridge were changed.

3. A 0–5 l $min^{-1}$ Wisa diaphragm air pump was used instead of the original pump. The pump was connected to a variable voltage supplier.

4. A flow meter of range 0–2.5 l $min^{-1}$ was used.

5. The original flow system was modified and the final design is shown in Fig. 1.

The sampled air is drawn into the glass flow system by the air pump (L) and enters through the bottom of the absorber

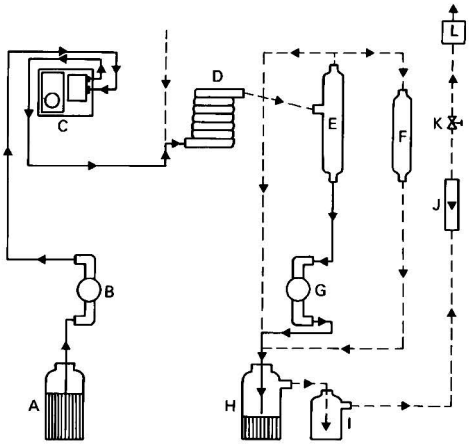


**Fig. 1.** Modified flow system. Solid line, solution flow; and broken line, air flow. A, Solution reservoir; B, reference optical cell; C, solution metering pump, 0–2 ml $min^{-1}$; D, 7-turn concurrent absorber; E and F, separators; G, sample optical cell; H, solution waste reservoir; I, trap; J, flow meter, 0–2.5 l $min^{-1}$; K, air flow control valve; and L, air pump, 0–5 l $min^{-1}$

(D), where chlorine reacts with the reagent. The direction of the air flow is the same as that of the reagent flow through the absorber. After the absorber, the air passes through separator E, where it is separated from the reagent and then drawn through a solution waste container (H), trap (I), flow meter (J), sample control valve (K) and out through the air pump.

The solution is pumped from the reagent reservoir (A) through the reference optical cell (B) into the absorber, where the chemical reaction occurs. It is then drawn through separator E to the sample optical cell (G). Separator F provides a constant-pressure balance for smooth flow through the sample optical cell. From the optical cell it is drawn into the waste reservoir (H).

### Preparation of Scrubbers

Coarse colourless silica gel crystals (BDH Chemicals) were initially saturated with doubly distilled water. The saturation was carried out slowly, to prevent breakage of the crystals. The system consisted of a midget impinger bubbler, which contained 20 ml of doubly distilled water, and was connected in series with two glass tubes. The first tube contained the colourless silica gel and the second contained dry blue silica gel. Air was drawn through the system at a flow-rate of about 200 ml $min^{-1}$ until the blue silica gel crystals changed colour. The following scrubbers were prepared.

A. A 30-g amount of the wet colourless silica gel crystals was immersed in 20 ml of solution containing 8.5 g of chromium trioxide (BDH Chemicals) and 3.5 g of concentrated sulphuric acid (BDH Chemicals). The impregnated silica gel crystals were packed into a U-tube of 23 mm i.d. and 150 ml volume and dried overnight in an oven at 80 °C, by a stream of dry air.

B. A 50-g amount of the wet colourless silica gel was immersed in a solution containing 10 g of chromium trioxide, 10 g (5.9 ml) of 85% orthophosphoric acid (Riedel-de Haen) and 10 ml of doubly distilled water. The silica gel crystals were then dried overnight at 70 °C, as described for scrubber A.

### Efficiency of Scrubber A in Removing Reducing Sulphur Compounds

The efficiency of scrubber A at temperatures in the range 23–60 °C in removing $SO_2$, $CH_3SH$, $C_2H_5SH$ and $H_2S$ was

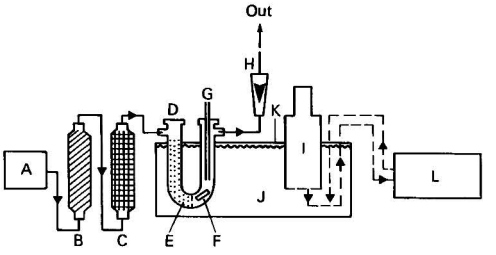


**Fig. 2.** PT calibration system. Solid line, air flow; and broken line, water flow. A, Air pump; B, silica gel air drier; C, activated charcoal filter; D, U-tube; E, glass beads; F, PT; G, thermometer; H, flow meter; I, thermoregulator, a heater and a water pump (Haake Model E2); J, water-bath; K, polyethylene foam cover; and L, Haake DK12 cooling system

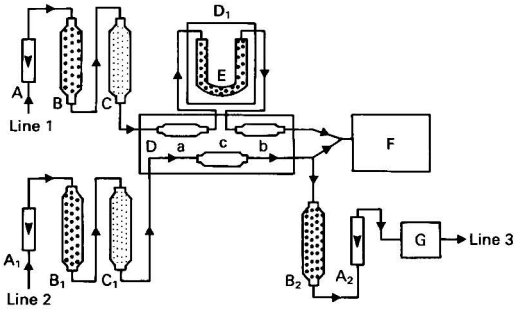


**Fig. 3.** Experimental flow system used to study the efficiency of scrubber A in removing reducing sulphur compounds. A,A1 and A2, flow meters; B,B1 and B2, charcoal filters; C and C1, silica gel air drier; D and D1, ovens; E, chromic acid scrubber; F, Beckman Acralyzer; G, air pump; and a, b and c, glass tubes for PTs

studied. Known concentrations of the reducing sulphur compounds and of $Cl_2$ were obtained by using Metronics permeation tubes (PTs).

Sulphur compound PTs were calibrated gravimetrically as follows. The calibrating system (Fig. 2) was constructed from a 9-l water-bath (J) covered with polyethylene foam (K), which isolated it from the surroundings. A system (I) (Haake Model E2) consisting of a thermoregulator, a heater and a water pump and also a cooling system (L) (Haake Model DK 12) were used. A two-chamber glass U-tube (D) (20 cm in height, 2 cm in diameter) having an inlet and an outlet was housed in the water-bath. The two parts of the U-tube were separated by a glass disc, having a few holes that enabled the air to flow through. One chamber was filled with glass beads (E) and the other contained a PT (F) and a thermometer (G).

Air was pumped through dried silica gel (B), activated charcoal (C), the U-tube and out through a flow meter (H). The temperature measured within the U-tube was accurate to within ±0.05 °C.

Calibration of $H_2S$ and thiol PTs was carried out just before and after the experiments, by measuring the descrease in mass during certain time periods, at the same temperature of the experiment.

Fig. 3 illustrates the system used for the efficiency measurements. The air flows through lines 1 and 2 were combined at the entrance to the analyser (F). The two lines were connected to the instrument through a tee. All connections in the system were made of glass or short PTFE tubing. Glass tubes a, b and c in which the PTs were placed were thermostatically controlled throughout the range 20–40 °C to within ± 0.05 °C. The temperature of the scrubber (E) was

thermostatically controlled between room temperature and 60 °C to within ±1 °C. The chlorine concentration was regulated by three parameters: the emission rate of $Cl_2$ from the PT (which was controlled by the thermostatic bath temperature), the rates of air flows through lines 1 and 2 and the rate of air flow bypassed through line 3. The concentrations of sulphur compounds were adjusted by the air flows through lines 1 and 2 and by the thermostatic bath temperature.

Each experiment included the following steps: (1) a, b and c without PTs; (2) a and b without PTs, c containing a $Cl_2$ PT; (3) b containing a sulphur compound PT, a containing no PT and c containing a $Cl_2$ PT; and (4) sulphur compound PT in a, $Cl_2$ PT in c, b containing no PT.

The instrument was zeroed by following step 1 and by changing the gain of the recorder. The span was adjusted according to step 2 and by changing the gain of the instrument. $Cl_2$ concentrations were measured throughout step 2. The percentage of sulphur compound interference was calculated from steps 2 and 3. The scrubber efficiency was determined using steps 2 and 4.

The experiments were carried out at a solution flow-rate of 2 ml $min^{-1}$ and an air flow-rate of 2 l $min^{-1}$. The sensitivity was 0.5 p.p.m. full-scale.

### HCl Interference in the Presence of the Scrubbers and at Different Relative Humidities

The flow system shown in Fig. 3 was also used to study HCl interference in the presence of scrubbers A and B. A hygrometer was housed in a glass tube between the scrubber (E) and glass tube (b) (Fig. 3). The solution and air flow-rates were 1 ml $min^{-1}$ and 2 l $min^{-1}$, respectively. The sensitivity was 0.25 p.p.m. *V/V* full-scale. Two groups of experiments were conducted as follows.

1. A few experiments were carried out with scrubbers A and B in the absence of $Cl_2$ at a room temperature of 26 °C. Air was sampled through dry silica gel, and the dry scrubber was maintained at 57–69 °C. Under these conditions it was found that the relative humidity achieved was 10%.

2. Other experiments were carried out with scrubber B in the presence of $Cl_2$. These were performed at a room temperature of 24–25 °C and scrubber temperatures in the range 25–53 °C. In these experiments the silica gel in line 1 was disconnected. Some of the experiments were carried out at room relative humidity (58%) and some at a relative humidity close to saturation at room temperature. Saturation was achieved by (*i*) replacing the silica gel tube with an impinger containing distilled water and (*ii*) bubbling air through the system overnight in order to achieve the proper conditions for the HCl PT and the scrubber.

The experimental steps were the same as those described above.

### Effect of $O_3$ on $Cl_2$ Measurements

In order to study $O_3$ interference, certain changes were made to the system shown in Fig. 3. Line 1 was changed as follows: air was drawn to the analyser through an $O_3$ generator and a scrubber of glass beads about 5 mm in diameter. The glass beads were packed in a U-tube of 23 mm i.d. and 150 ml volume. The scrubber was thermostatically controlled in the range 23.5–80 °C. The $O_3$ concentration in these experiments was 0.70 p.p.m. at the mixing point of the two flows. The work was performed at $[O_3]$ to $[Cl_2]$ ratios of 1.6 and 2.4.

Another means of removing $O_3$ was to add 20 g $l^{-1}$ of $NaNO_2$ to the absorbing solution. The system was the same as before, but without the glass beads scrubber. The $O_3$ concentration at the mixing point of the flows was 0.10 p.p.m. and the $[O_3]$ to $[Cl_2]$ ratio was 2.3.

### Effect of Humid Scrubber B on $Cl_2$ Measurements

Line 1 in Fig. 3 was used in order to perform these experiments and line 2 was disconnected. The experiments were carried out under three humidity conditions and at different scrubber temperatures in the range 25–69 °C. Different periods of conditioning of the scrubber were used prior to each experiment (Table 3). The relative humidity was measured as described under HCl Interference in the Presence of Scrubbers and at Different Relative Humidities.

The three humidity conditions used were as follows. (a) The sampled air was saturated with water vapour at a room temperature of 25 °C. The saturation was achieved by bubbling the air through a midget impinger containing 20 ml of doubly distilled water. The impinger was located in place of the silica gel tube. (b) Air at a room relative humidity of 65% and a room temperature of 25 °C was used. The silica gel tube at line 1, Fig. 2 was disconnected. (c) Air at a room temperature of 25 °C was sampled through dry silica gel, placed before the entrance to the scrubber. The relative humidity obtained at this temperature was 10%. The air flow-rate in all experiments was 2 l $min^{-1}$.

## Results and Discussion

### Performance of the Analyser

The modifications that were carried out on the Beckman Acralyzer Model K-1008 enabled it to be used for chlorine monitoring. The sensitivity of the instrument was increased by changing its electronic system and by increasing the air flow. The new pump produced a more stable air flow than the original one. Originally it was connected to an exit from separator F, whereas in the modified system this exit was closed and the air pump was connected to the waste solution container through a trap. This reduced the solution level in separator E (Fig. 1), lowering the lag time to about one third of the original (at a solution flow-rate of 2 ml $min^{-1}$ it was about 10 min). The sensitivity achieved was 0.25 p.p.m. full-scale at a solution flow-rate of 1 ml $min^{-1}$ and an air flow-rate of 2 l $min^{-1}$. Other desired sensitivities could be achieved by changing the solution and air flow-rates.

It should be noted that rather than using the Beckman Acralyzer within the system, other instruments, manufactured by other companies, which are more compact and have smaller lag times, could be used. This may alleviate the necessity to modify the electronics and the flow systems.

### Efficiency of Scrubber A in Removing Reducing Sulphur Compounds

Scrubbers consisting of pumice or silica gel as the absorbent, impregnated in a solution containing chromium trioxide and sulphuric or phosphoric acid, have been tried. A preliminary investigation was carried out on scrubbers made of pumice. They were found to become humid within a short time, causing absorption of the chlorine. The chlorine concentration monitored rapidly decreased to zero, especially at low chlorine concentrations. Scrubbers A and B (see Experimental) were the most efficient, and more resistant to humidity.

In order to determine the efficiency of the scrubber, $Cl_2$ was monitored first in the absence of sulphur compounds. In the next stage, measurements were carried out in the presence of these compounds, with and without the scrubber.

If the interference of sulphur compounds in chlorine measurements is due only to the direct reaction between the two, the interference will be given by the percentage of chlorine that reacts. The amount of chlorine that reacts with sulphur compounds in a stationary state will be dependent on the stoicheiometry of the reaction, the concentration of each one of the two, the rate constant of the chemical reaction and also on the time from the moment they enter the solution until

**Table 1.** Interference of sulphur compounds and removal efficiency of scrubber A

| Experiment No. | Sulphur compound (S) | $Cl_2$ concentration,* p.p.m. | S concentration,* p.p.m. | Ratio of concentrations, [S]:[$Cl_2$] | Scrubber temperature/°C | Absorbance in the presence of $Cl_2$ and in the absence of S | Absorbance in the presence of $Cl_2$ and S: Without scrubber A | Absorbance in the presence of $Cl_2$ and S: With scrubber A | Interference, % | Efficiency of scrubber A, % |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $SO_2$ | 0.458 | 0.110 | 0.24 | 23 | 0.90 | 0.57 | 0.86 | 36.7 | 96 |
| 2 | $SO_2$ | 0.165 | 0.136 | 0.82 | 54 | 0.320 | 0.182 | 0.335 | 43.1 | 100 |
| 3† | $SO_2$ | 0.145 | 0.127 | 0.88 | 59 | 0.275 | 0.105 | 0.275 | 61.8 | 100 |
| 4 | $SO_2$ | 0.111 | 0.155 | 1.40 | 23 | 0.220 | 0.035 | 0.220 | 84.0 | 100 |
| 5 | $CH_3SH$ | 0.221 | 0.078 | 0.35 | 57 | 0.45 | 0.000 | 0.45 | 100 | 100 |
| 6 | $C_2H_5SH$ | 0.536 | 0.188 | 0.35 | 60 | 1.06 | 0.000 | 1.06 | 100 | 100 |
| 7 | $C_2H_5SH$ | 0.159 | 0.188 | 1.18 | 60 | 0.300 | 0.000 | 0.300 | 100 | 100 |
| 8 | $H_2S$ | 0.186 | 0.813 | 4.40 | 23 | 0.380 | 0.000 | 0.380 | 100 | 100 |

* The concentrations were calculated after mixing of the two flows (lines 1 and 2, Fig. 3).
† In this experiment the air drawn through the scrubber was not dried over silica gel.

they reach the optical cell. For certain conditions of reagent flow and geometry of the flow system, this time is constant. If the stoicheiometry of the reaction is 1:1 and the reaction is complete, the interference will be directly dependent on the relative concentration of the sulphur compound to that of chlorine.

The results of experiments 1–4 in Table 1 show that an increase in the [$SO_2$] to [$Cl_2$] ratio causes a large decrease in the absorbance. With thiols it seems that more than one equivalent of chlorine reacts with each equivalent of thiol. No $Cl_2$ could be detected in the presence of thiols and hydrogen sulphide at the concentrations indicated in Table 1. In all the experiments the efficiency of scrubber A was close to 100%, independent of the scrubber temperature. A qualitative experiment carried out on $SO_2$ with scrubber B showed the same behaviour as that of scrubber A.

### Effect of Scrubbers on Oxidation of HCl During $Cl_2$ Monitoring

During the sampling of air containing 10.3 p.p.m. of HCl and no $Cl_2$, it was found qualitatively that some oxidation of HCl occurred when using scrubber A. No such oxidation occurred when scrubber B was used.

Monitoring of 0.17 p.p.m of $Cl_2$ in the presence of 10.3 p.p.m. of HCl, as described under Experimental, was carried out using scrubber B. The results show no difference, or only a slight decrease, in the concentrations observed, compared with those obtained in the absence of HCl. This slight decrease was within the experimental error. However, no increase in concentration was obtained. Hence no oxidation of HCl occurred even at very high concentrations of HCl when scrubber B was used.

### Effect of $O_3$ on $Cl_2$ Monitoring

The interference of $O_3$ in the presence of a glass-beads scrubber was studied in two separate experiments, as described under Experimental. The results show that while the glass-beads scrubber was at room temperature no $Cl_2$ could be detected in the presence of $O_3$. When the temperature was increased a rapid rise in $Cl_2$ concentration was observed. At 50 °C about 80% of the value obtained in the absence of $O_3$ was retained. Above 50 °C an additional but slower rise in $Cl_2$ concentration as a function of temperature increase was observed. An optimum value of about 90% of the "original" reading was obtained at temperatures of 60–65 °C. At higher temperatures a slow decrease in those values was observed.

**Table 2.** Efficiency of a solution containing $NaNO_2$ in removing $O_3$

| Concentration ratio, [$O_3$]:[$Cl_2$] | $NaNO_2$ present | Absorbance |
|---|---|---|
| 0 | No | 0.56 |
| 0 | Yes | 0.56 |
| 0.23 | No | 0.49 |
| 0.23 | Yes | 0.56 |

**Table 3.** Effect of humid scrubber on $Cl_2$ monitoring

| Experiment No. | Relative humidity at 25 °C* | Scrubber temperature/°C | Conditioning period of the scrubber†/h | Decrease in $Cl_2$ concentration in the presence of scrubber B,% |
|---|---|---|---|---|
| 1 | a | 33 | 16 | 45 |
| 2 | a | 44 | 40 | 10 |
| 3 | a | 45 | 2 | 3 |
| 4 | a | 69 | 70 | 2 |
| 5 | a | 69 | 50 | 0 |
| 6 | b | 40 | 4 | 5 |
| 7 | b | 69 | 75 | 0 |
| 8 | c | 25 | 200 | 0 |
| 9 | c | 69 | 500 | 0 |

* The description of relative humidity a, b, and c is given under Effect of Humid Scrubber B on $Cl_2$ Measurements in the Experimental section.
† Period during which the scrubber was in contact with the sampled air under the experimental conditions before the beginning of the experiment.

As mentioned in a previous paper,[2] the addition of $NaNO_2$ to the absorbing solution was also useful in preventing $O_3$ interference. An experiment showing the efficiency of such a solution in a monitoring system was carried out. The results are given in Table 2.

### Effect of Humid Scrubber B on $Cl_2$ Monitoring

Table 3 shows that as the temperature of scrubber B increases, the interference of humid air is decreased. At high relative humidity and a low scrubber temperature (experiment 1), the $Cl_2$ concentration decreased by 45%. At such a low scrubber temperature, conditioning for 16 h was sufficient to increase the water content in the silica gel to a level that caused a significant absorption of $Cl_2$. When the temperature of the scrubber was increased to 44 °C the interference was de-

creased to only 5%, even though the conditioning time was 40 h (experiment 2). At 69 °C the interference found was close to zero, even when the conditioning time was as long as 70 h (experiments 3 and 4).

At a lower relative humidity (experiments 6 and 7), the interference decreased. At a very low relative humidity the scrubber did not interfere even at low temperatures and with long conditioning periods.

## Conclusion

The spectrophotometric method based on the reaction of chlorine with 4-nitroaniline can be used for the continuous monitoring of chlorine in air.

Reducing sulphur compounds can be removed efficiently using a scrubber prepared from chromic acid absorbed in silica gel. A chromic acid scrubber prepared from chromium trioxide and phosphoric acid has a high capacity and a high resistance to humidity when heated to 69 °C. $O_3$ can be removed either by a glass-beads scrubber heated to 60–65 °C or by the addition of $NaNO_2$ to the absorbing solution.

## References

1. Langhorst, M. L., *Am. Ind. Hyg. Assoc. J.*, 1982, **43**, 347.
2. Gabbay, J., Davidson, M., and Donagi, A. E., *Analyst*, 1976. **101**, 128.
3. Saltzman, B. E., and Wartburg, A. F., *Anal. Chem.*, 1965, **37**, 779.
4. Lindqvist, F., *Analyst*, 1972, **97**, 549.
5. Schulze, F., *Anal. Chem.*, 1966, **38**, 748.
6. Wartburg, A. F., Brewer, A. W., and Lodge, J. P., *Int. J. Air Water Pollut.*, 1964, **8**, 21.

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# Morpholine as an Absorbing Reagent for the Determination Of Sulphur Dioxide

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Several methods have been developed for the determination of sulphur dioxide in the environment, although some involve the use of hazardous mercury salts. A method is described of trapping sulphur dioxide by forming an adduct with morpholine. Sulphur dioxide is determined in the adduct using *p*-rosaniline hydrochloride, which forms a coloured complex with maximum absorption at 560 nm. The method is sensitive and a 0.025 μg ml$^{-1}$ concentration of sulphur dioxide can be determined in solution. Interferences from Cu(II), Pb(II), Mn(II), Fe(III), Cr(III), V(V), nitrite and hydrogen sulphide have been studied. In addition concentrations of morpholine thrown into the atmosphere by this method have been investigated to assess any possible hazards due to the use of morpholine as a trapping agent during the sampling process.

**Keywords:** *Sulphur dioxide determination; morpholine; spectrophotometry; p-rosaniline hydrochloride; formaldehyde*

A literature survey reveals that a considerable amount of work has been carried out on the development of various physico-chemical techniques for the determination of trace amounts of sulphur dioxide—a compound which may be harmful to animals and plants.[1,2]

The spectrophotometric determination of sulphur dioxide is a useful technique owing to its simplicity and sensitivity and many research papers are available on the subject.[3–8] Most of the methods use mercury(II) salts either for fixation or for chemical reactions in which excess of mercury(II) salts are utilised for the determination of sulphur dioxide.[3–5,8] Only a few methods that avoid the use of poisonous mercury(II) salts are available.[6,7,9–11]

Sulphur dioxide is known to be absorbed by many organic substances.[9–16] Sulphur dioxide forms an adduct with morpholine and its derivatives.[13,15] This aspect is employed in this work in which morpholine is used as a trapping solution for sulphur dioxide. Sulphite or sulphurous acid is used as a source of sulphur dioxide and is added to morpholine. The product obtained is treated with acid bleached *p*-rosaniline hydrochloride and formaldehyde to yield a violet colour with an absorption maximum at 560 nm. The intensity of the colour is proportional to the concentration of sulphur dioxide and obeys Beer's law. Sulphur dioxide in solution at a concentration as low as 0.025 μg ml$^{-1}$ can be determined by this method.

## Experimental

### Apparatus

A Hilger and Watts UV H 700 spectrophotometer with 1 cm cells was used for the absorbance measurements.

### Reagents

All chemicals used were of analytical-reagent grade. Doubly distilled water was used for preparing all the solutions.

*Sodium sulphite solution.* A 0.1 g mass of anhydrous sodium sulphite was dissolved in distilled water and the volume was made up to 250 ml. This solution was standardised iodimetrically and diluted further to obtain a 4 μg ml$^{-1}$ solution of sodium sulphite. Fresh solutions were prepared before each experiment as sodium sulphite is unstable in solution.

*Sulphurous acid.* Sulphur dioxide was prepared by the action of concentrated hydrochloric acid on sodium sulphite and passed through distilled water. The sulphurous acid thus formed was determined conductimetrically after oxidation to sulphuric acid using hydrogen peroxide and was further diluted to obtain a 2 μg ml$^{-1}$ solution.

*Morpholine solution.* Morpholine (4 cm$^3$) was diluted to 1000 ml with distilled water.

p-*Rosaniline hydrochloride solution.* A 100-mg mass of the *p*-rosaniline hydrochloride was dissolved in distilled water, de-colourised with 15 ml of concentrated hydrochloric acid and diluted to 250 ml.

*Formaldehyde solution.* A 2.5-ml volume of 40% formaldehyde was made up to 500 ml with distilled water.

*Sulphamic acid*, 0.6%. The solution was prepared freshly.

### Procedure

A 10-ml volume of morpholine solution was pipetted into a series of 50-ml calibrated flasks. Volumes of 2, 4, 6, 8 and 10 ml of sodium sulphite or sulphurous acid were then pipetted into these flasks. The flasks were tightly stoppered and thoroughly shaken. This was followed by the addition of 10 ml each of *p*-rosaniline hydrochloride and formaldehyde solutions. The flasks were well shaken and the volume was made up to 50 ml. The pH of the solution was noted and the absorbances were measured at 560 nm against a blank after 20 min. The absorbances were plotted against the concentration of sulphite - sulphur dioxide to obtain the calibration graph.

## Results and Discussion

It is well known that sulphur dioxide forms clathrates and charge-transfer complexes with organic substances.[9,12–16] In this work sulphur dioxide - sulphite was trapped in basic morpholine solution to form the adduct and the sulphur dioxide in the adduct was determined with acidified *p*-rosaniline hydrochloride.

### Effect of pH

The effect of pH on the reaction of the morpholine - sulphur dioxide adduct with *p*-rosaniline hydrochloride and formaldehyde was studied. It was found that the intensity of the violet colour obtained by the reaction was enhanced with an increase in pH. The colour intensity of the dye was also pH dependent. The effect of pH on the intensity of the colour of the dye was studied with the reagent blank and it was found that the change in absorbances due to dye was at a minimum when the pH of the final solution was between 0.8 and 1.0

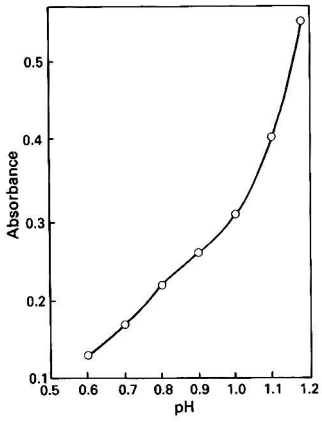


**Fig. 1.** Effect of pH on the reagent blank

(Fig. 1). The acid present in *p*-rosaniline hydrochloride not only helps in releasing sulphur dioxide but also in achieving a pH of 0.8–1.0.

### Beer's Law and Reproducibility

Beer's law was obeyed in the concentration range 1.25–20 μg of sulphur dioxide in 50 ml of solution. The method was found to be reproducible and the standard deviation was found to be 0.006.

### Comparison of Morpholine with Sodium Tetrachloromercurate(II) for Trapping Sulphur Dioxide

The validity of the proposed method was checked by using sodium sulphite and sulphurous acid, whose sulphur dioxide content had been determined by the West - Gaeke method. Care was taken to maintain the same pH in both the methods to avoid the variation in the absorbance of the solution owing to the effect of pH on the dye. It was observed that the absorbance was independent of the nature of the trapping solution. This is illustrated in Table 1.

The stability of sulphur dioxide or sulphite in morpholine was also investigated. Anhydrous sodium sulphite (50 mg) was added to 20 ml of morpholine solution and the volume was made to up 100 ml with doubly distilled water. Aliquots were taken and analysed for the sulphur dioxide content once a day for 15 days. It was found that the sulphur dioxide - sulphite content remained unchanged.

### Absorption Efficiency

Sulphur dioxide, prepared by the action of hydrochloric acid on sodium sulphite, was collected in a Corning glass sampler (500 ml) provided with a septum arrangement to draw known amounts of sulphur dioxide. Using this gas, a dilute sample solution with a sulphur dioxide concentration of 15 μg $ml^{-1}$ was prepared. A 1-ml volume of the gas (15 μg $ml^{-1}$) was directly injected into 10 ml of 0.4% morpholine using a gas-tight syringe. In another experiment 1 ml of the gas (15 μg $ml^{-1}$) was injected into a gas sampler of 500 ml capacity, which was initially flushed with pure dry nitrogen. The sulphur dioxide - nitrogen mixture in the gas sampler was passed through two midget inpingers (25 ml capacity) containing 10 ml of 0.4% morpholine connected in series using pure nitrogen as a carrier gas at a flow-rate of 0.5 l $min^{-1}$ for 30 min. These impingers are similar to the midget impingers of Type 9700 of Ace Glass Company.[18] The sulphur dioxide determined in both the impingers indicated that the first impinger contained 100% sulphur dioxide, which was comparable to the concentration of sulphur dioxide obtained by directly injecting 1 ml into 10 ml of 0.4% morpholine. No sulphur dioxide was found in the second impinger.

**Table 1.** Comparison of the morpholine adduct method with the West - Gaeke method

| | Absorbance at 560 nm | |
|---|---|---|
| Amount of sulphur dioxide added/μg | West - Gaeke method | Morpholine adduct method |
| 3.24 | 0.022 | 0.023 |
| 6.47 | 0.051 | 0.044 |
| 9.71 | 0.076 | 0.076 |
| 12.94 | 0.099 | 0.099 |
| 16.18 | 0.130 | 0.130 |

### Application of the Method

To extend the method to the determination of sulphur dioxide in air, laboratory air was passed through 10 ml of 0.4% morpholine and through sodium tetrachloromercurate(II) using two separate flow meters. The sulphur dioxide concentration determined by both the methods was found to be 0.08 p.p.m.

### Effect of Foreign Ions

The interference of nitrite was investigated by adding 10–50 μg of nitrite ions to 10 ml of absorbing solution containing 30 μg of sulphite and the absorbances were measured as explained under Procedure. It was observed that the intensity of the coloured complex was diminished in the presence of nitrate. To eliminate this error, 2 ml of 0.6% sulphamic acid were added to the absorbing solution before the addition of nitrite and sulphite. The colour was developed and measured and the absorbance was found to be slightly lower than the expected value. The error was reduced by introducing a separate trap containing 0.6% sulphamic acid instead of adding sulphamic acid to the absorbing solution as suggested by Paul and Gupta.[17]

The sulphide ion, which is also present in the atmosphere mainly as hydrogen sulphide, interferes in the proposed method and to study its effect,16 μg of sulphide ion were added to 10 ml of 0.4% morpholine solution containing 18 μg of sulphur dioxide. These amounts, if present in 40 l of air, correspond to 0.16 p.p.m. of sulphur dioxide and 0.28 p.p.m. of sulphide. The colour was developed as described under Procedure. It was found that the sulphide ion enhanced the absorbance; the content of sulphur dioxide was found to be 0.24 p.p.m. compared with the actual value of 0.16 p.p.m. The actual concentration of hydrogen sulphide in the environment is generally too low to cause any significant variation in the sulphur dioxide value.

The influence of metallic ions on this method was studied by adding 10–50 μg of Cu(II), Pb(II), Mn(II), Fe(III), Cr(III) and V(V) to 10 ml of absorbing solution containing sulphite before the colour development. It was observed that 20 μg of Cu(II), 50 μg of Pb(II), 50 μg of Fe(III) and 50 μg of V(V) did not interfere. Mn(II) interfered even when only 10 μg were added. Although it is not expected that the metallic impurity in the atmosphere is as high as the concentrations tested in this work, we have studied the effects of higher concentrations of metallic ions to simulate possible industrial situations. The addition of the sodium salt of ethylenediaminetetraacetic acid (2 ml of 0.066%) masks 100 μg of metallic ions without affecting the determination of sulphur dioxide.

### Morpholine Concentration in the Atmosphere

The threshold limit of morpholine in air is 20 p.p.m.[19] When air is passed through morpholine during sampling it is expected that the morpholine vapour will contaminate the atmosphere. Hence, it is essential to study the loss of morpholine from 10 ml of 0.4% morpholine, being used as the absorbing reagent in the determination of atmospheric sulphur dioxide.

A 40-l volume of air was passed through 10 ml of 0.4% morpholine at a rate of 0.5 l $min^{-1}$. The morpholine content in the solution was determined by adding an excess of 0.1 N hydrochloric acid (5 ml) and titrating the excess of acid with 0.02 M sodium hydroxide solution using methyl red as an indicator. The difference in the morpholine content between the two samples, one of them through which 40 l of air was passed and the other which was not subjected to bubbling of air, was 1 mg, which corresponds to 3.7 p.p.m. in air. When a similar experiment was conducted by passing 375 l of air, the loss of morpholine was found to be 2 mg, which corresponds to 1.4 p.p.m. of morpholine in air. These results indicate clearly that the loss of morpholine during the sampling process is very much below the threshold limit and poses no hazardous effects.

## Conclusion

Sulphur dioxide - sulphite is trapped in morpholine solution to give an adduct that, when treated with *p*-rosaniline hydrochloride and formaldehyde solutions, yields a violet colour with an absorption maximum at 560 nm. This investigation avoids the use of poisonous mercury(II) salts and is as sensitive as the West - Gaeke method. A sulphur dioxide content as low as 0.025 μg $ml^{-1}$ in solution can be determined by this method. Morpholine is very effective as an absorbent for sulphur dioxide as the adduct formed is stable. The morpholine concentration passing into the atmosphere during the sampling process is much below the threshold limit.

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## References

1. Marr, I. L., and Cresser, M. S., "Environmental Chemical Analysis," Thomson Litho, East Kilbride, 1983, p. 250.
2. Sharma, H. C., and Rao, D. N., *Indian J. Environ. Health*, 1983, **25**, 169.
3. Hize, W. K., Kippenberger, D. J., and Humphrey, R. E., *Microchem. J.*, 1973, **20**, 43.
4. Humphrey, R. E., Ingram, G. S., and Crump, D. K., *Microchem. J.*, 1982, **27**, 351.
5. Okutani, T., and Utsumi, S., *Bull. Chem. Soc. Jpn.*, 1967, **40**, 1386.
6. Ramakrishna, T. V., and Balasubramaniyan, N. B., *Indian J. Chem.*, 1982, **21**, 217.
7. Raman, V., Singh, M., and Parashar, D. C., *Microchem. J.*, in the press.
8. West, P. W., and Gaeke, G. C., *Anal. Chem.*, 1956, **28**, 1816.
9. Bhatt, A., and Gupta, V. K., *Analyst*, 1983, **108**, 374.
10. Christopher, R. F., and Barry, D., *Environ. Sci. Technol.*, 1982, **16**, 62.
11. DasGupta, P. K., Decesare, K., and Ullrey, J. C., *Anal. Chem.*, 1980, **52**, 1912.
12. Grundnes, G., and Christian, S. D., *Acta Chem. Scand.*, 1969, **23**, 3583.
13. Harris, P. K., and Spragg, R. A., *Org. Magn. Reson.*, 1969, **1**, 329.
14. Kanamueller, J. M., *J. Inorg. Nucl. Chem.*, 1971, **33**, 4051.
15. Sweeney, N. P., and Thom, K. F., *U.S. Pat.*, 3943 146; *Chem. Abstr.*, 1976, **85**, 5637c.
16. Yamaguchi, T., Kawasaki, T., and Emori, S., *Kobunshi Kagaku*, 1971, **28**, 336; *Chem. Abstr.*, 1971, **75**, 152190y.
17. Paul, K. R., and Gupta, V. K., *Atmos. Environ.*, 1983, **17**, 1773.
18. Pagnotto, L. D., "Gas Vapor Sample Collectors," *in* "Air Sampling Instruments For Evaluation of Atmospheric Contaminants," Fifth Edition, American Conference of Governmental Industrial Hygienists, Cincinnati, OH, 1978, R7.
19. Hawley, G. G., "Condensed Chemical Dictionary," Ninth Edition, Van Nostrand Reinhold, New York, 1977, p. 591.

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# Kinetic - Catalytic Determination of Cobalt by Oxidation of Pyrogallol Red by Hydrogen Peroxide

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A kinetic - spectrophotometric method for the determination of trace amounts of cobalt(II) based on its catalytic effect on the hydrogen peroxide oxidation of Pyrogallol Red (PGR) is proposed. Under optimum conditions, cobalt can be determined in the range 0.20–13.00 ng ml$^{-1}$. The limit of detection is 0.16 ng ml$^{-1}$ of Co and the limit of quantitation is 0.54 ng ml$^{-1}$ of Co. The precision and accuracy of the method using a fixed time of 5 min were also determined. The influence of 18 foreign species is discussed.

**Keywords:** *Cobalt determination; catalytic oxidation; Pyrogallol Red; hydrogen peroxide; kinetic - spectrophotometric method*

The catalytic action of cobalt may be observed in the oxidation of various substituted phenols with hydrogen peroxide. This effect is especially great if the phenols contain hydroxy groups in the *ortho* position,[1] and some cobalt-catalysed hydrogen peroxide reactions, such as those with alizarin,[2] 9-phenyl-3,4,7-trihydroxyfluorone,[3] Tiron,[4,5] haematoxylin,[6] catechol[7–9] and 4-(4-nitrophenylazo)catechol,[10] have been described. There are some papers, however, that describe reactions with *meta*-substituted phenols such as 4-(4-nitrophenylazo)resorcinol[11] and with *para*-substituted phenols such as Alizarin Red S.[12,13]

The catalytic effect of cobalt on the oxidation of Pyrocatechol Violet[14] and Bromopyrogallol Red,[15] dyes of the triphenylmethane series, have been studied. In relation to Bromopyrogallol Red, it is possible to carry out the determination of the catalyst in the range $7.76 \times 10^{-10}$–$38.80 \times 10^{-10}$ g cm$^{-3}$ of Co in alkaline media.

We earlier carried out a kinetic study of the uncatalysed reaction between Pyrogallol Red (PGR) and hydrogen peroxide and the cobalt(II)-catalysed hydrogen peroxide oxidation of PGR.[16] This paper reports the analytical application of the reaction.

## Experimental

### Reagents

All reagents used were of analytical-reagent grade unless specified otherwise.

*Cobalt(II) stock solution*, 500 mg l$^{-1}$. A 1.247-g amount of cobalt(II) nitrate [$Co(NO_3)_2.6H_2O$, Merck] was dissolved in distilled water and diluted to 1 l. The solution was standardised with EDTA. A 50 ng ml$^{-1}$ working solution was prepared by appropriate dilution of the stock solution.

*Hydrogen peroxide stock solution*, 30% *m/V*. This was standardised by permanganate titration.

*Buffer solution*, 0.1 M. Sodium tetraborate - 0.02 M hydrochloric acid, pH 8.

*Pyrogallol Red solution*, $8 \times 10^{-4}$ M. Dissolve 0.160 g of PGR (Merck) in 500 ml of methanol. This solution is stable for at least 1 month.

### Apparatus

All spectrophotometric measurements were made on a Shimadzu UV-240 spectrophotometer with an OPI-2 coupled optional program unit. A Crison-501 pH meter, equipped with a Metrohm EA-121 Ag - AgCl electrode system, was used for measuring the pH of the solutions. The pH should be measured to an accuracy of ±0.01 pH unit. A Frigedor Selecta-396 refrigerator unit for use in a water-bath and a Thermotronic Selecta-389 immersion thermostat capable of maintaining the temperature to within ±0.05 °C were also used. All the solutions were previously heated to the working temperature (25 ± 0.05 °C) in a thermostat and this temperature was maintained in the reaction cell during the experiment.

## Results and Discussion

### Optimum Conditions for the Cobalt-catalysed Reaction

The kinetic study of the Co(II)-catalysed hydrogen peroxide oxidation of PGR in alkaline medium (pH 8.3) resulted in the following rate equation because the uncatalysed reaction does not take place and the catalysed reaction is zero order with respect to PGR[16]:

$$v = -\frac{d[PGR]}{dt} = k_{total}[H_2O_2][Co][B_4O_7^{2-}]^{-1}$$

The optimum conditions should be selected such that the maximum sensitivity and the largest linear range are obtained together with the maximum correlation coefficient and precision. In order to find these optimum working conditions, the influence of reagent concentration was initially studied. The procedure and the order of addition of reagents have already been described.[16]

The kinetic data obtained for the dependence of the hydrogen peroxide concentration on the initial reaction rate show that the analytical sensitivity of the catalysed reaction increases with increasing hydrogen peroxide concentration up to $14 \times 10^{-2}$ M. The reproducibility, however, is not good, possibly owing to the decomposition of hydrogen peroxide, which takes place to a measurable extent. Therefore, $3.5 \times 10^{-2}$ M $H_2O_2$ was chosen as the recommended concentration as a compromise between reproducibility and sensitivity.

Borate has an inhibitory effect on the initial reaction rate. This effect may be caused by the formation of a complex between the dye and borate and also by the cobalt-complexing ability of borate. There is also a linear relationship between absorbance (measured at a fixed time of 5 min, and at the 515-nm maximum wavelength of PGR at pH 8.3) against borate concentration in the range $2 \times 10^{-2}$–$8 \times 10^{-2}$ M ($y = 0.531 + 1.86c_{B_4O_7^{2-}}$; $r = 0.998$). The optimum pH is 8.3, and to achieve this the reaction system must be buffered as effectively as possible. It was found that a borate concentration of $4 \times 10^{-2}$ M is the best compromise between this purpose and the inhibitory effect.

**Table 1.** Calibration graphs for the fixed-time method. Conditions as follows: PGR, $3.3 \times 10^{-5}$ M; $H_2O_2$, $3.5 \times 10^{-2}$ M; $Na_2B_4O_7$, $4 \times 10^{-2}$ M; pH, 8.3; $I$, 0.1 M; $\lambda = 515$ nm; and temperature, 25 °C

| Time/ min | Calibration graphs (0–13.0 ng ml$^{-1}$ of Co) | $r$ |
|---|---|---|
| 1.0 | $A = 0.7306 - 0.0119c$ | −0.992 |
| 3.0 | $A = 0.7277 - 0.0221c$ | −0.9989 |
| 5.0 | $A = 0.7274 - 0.0302c$ | −0.9998 |
| 10.0 | $A = 0.7256 - 0.0452c$ | −0.9988 |
| 15.0 | $A = 0.7167 - 0.0539c$ | −0.994 |

**Table 2.** Precision data for the method. Equation of calibration graph: $y = 0.7274 - 0.0302\,c$ ($r = -0.9998$). The following conditions were used: PGR, $3.3 \times 10^{-5}$ M; $H_2O_2$, $3.5 \times 10^{-2}$ M $Na_2B_4O_7$, $4 \times 10^{-2}$ M; pH, 8.3; $I$, 0.1 M; $\lambda = 515$ nm; and temperature, 25 °C

| True concentration of cobalt(II)/ ng ml$^{-1}$ | Mean concentration of cobalt(II) found/ ng ml$^{-1}$ | Standard deviation/ ng ml$^{-1}$ |
|---|---|---|
| 0.40 | 0.44 | 0.07 |
| 1.00 | 0.99 | 0.08 |
| 2.00 | 2.05 | 0.09 |
| 4.00 | 4.1 | 0.14 |
| 8.00 | 8.0 | 0.14 |
| 12.00 | 11.99 | 0.09 |

No change in the initial rate of reaction was detected with variation in PGR concentration in the range $1.6 \times 10^{-5}$–$4.8 \times 10^{-5}$ M. From the analytical point of view, a PGR concentration should be used that provides an absorbance in the range of minimum photometric error, and for this purpose $3 \times 10^{-5}$ M PGR was chosen as the most suitable concentration.

The analytical sensitivity of the catalysed reaction (*i.e.*, the slope of the calibration graph) was found to increase with increasing temperature; this would suggest that the temperature should be raised to 40 °C or even higher in order to optimise the method. With increasing temperature, however, the standard deviation increases. In addition, no dependence on temperature between 15 and 25 °C was noticeable for the uncatalysed reaction, but the initial reaction rate increased with increasing temperature in the range 30–40 °C. Therefore, 25 °C was chosen, as a compromise between precision and sensitivity (*i.e.*, the slope of the calibration graph and the highest ratio of the catalysed and uncatalysed reaction rates).

To summarise, the best experimental conditions for the catalytic determination of cobalt are $H_2O_2$ $3.5 \times 10^{-2}$ M, $Na_2B_4O_7$ $4 \times 10^{-2}$ M, PGR $3 \times 10^{-5}$ M, pH 8.3 and temperature 25 °C.

## Calibration Graphs

To obtain the calibration graphs, four methods were used: fixed-time, fixed-concentration, initial-rate and rate-constant methods. The best method for our catalysed reaction was chosen on criteria of sensitivity [*i.e.*, the slope ($S$) of the calibration graph], linear range and correlation coefficient ($r$).

### *Fixed-time method*

The calibration graphs of absorbance *versus* cobalt concentration at times of 1, 3, 5, 10 and 15 min are shown in Table 1. From these results, it can be seen that the slope increases with time. The best correlation coefficient was obtained for a fixed time of 5 min and this was chosen as the most suitable measuring time.

### *Fixed-concentration method*

The reciprocal of the time needed for the absorbance to decrease to 0.5500 (about 75% of the initial absorbance of the uncatalysed reaction) was plotted against cobalt concentration in the range 2.0–10.0 ng ml$^{-1}$. This calibration graph has the following equation: $1/t = -0.0014 + 0.0008c$ with $r = 0.997$, where the time was measured in seconds.

### *Initial-rate method*

A plot of the reaction rate (first 5 min) *versus* cobalt concentration yields the equation $v = 0.0008 + 0.0055c$ with $r = 0.9996$.

### *Rate-constant method*

The plots of log $A$ *versus* time (first 5 min) for cobalt concentrations in the range 0.2–13.0 ng ml$^{-1}$ were straight lines. From the slope ($K$) plotted against cobalt concentration the equation $K = -0.0015 + 0.0047c$ with $r = 0.995$ was obtained.

From the results obtained above, it can be inferred that the fixed-time method is the best as a better sensitivity, linear range and correlation coefficient were obtained.

## Limit of Detection and Limit of Quantitation

The theoretical limit of detection ($c_{LD} = K_d s_b/S$)[17,18] for a numerical factor $K_d = 3$ (confidence level) is 0.16 ng ml$^{-1}$ of Co at a fixed time of 5 min. The standard deviation ($s_b$) for 10 independent measurements with $3 \times 10^{-5}$ M PGR, $3.5 \times 10^{-2}$ M $H_2O_2$ and $4 \times 10^{-2}$ M $Na_2B_4O_7$ (uncatalysed reaction) is $1.62 \times 10^{-3}$ absorbance unit. The slope of the calibration graph is 0.0302 absorbance unit (ml ng$^{-1}$ of Co). The experimental limit of detection is 0.20 ng ml$^{-1}$ of Co.

The limit of quantitation ($c_{LQ} = K_q s_b/S$)[18] for a numerical factor $K_q = 10$ (confidence level) is 0.54 ng ml$^{-1}$ of Co. The regions of analyte measurement are as follows: analyte not detected, <0.16 ng ml$^{-1}$ of Co; region of detection, 0.16–0.54 ng ml$^{-1}$ of Co; and region of quantitation, > 0.54 ng ml$^{-1}$ of Co.

## Precision and Accuracy

A study of the precision was performed by carrying out 10 independent measurements on solutions of various concentrations of cobalt(II) and fixed concentrations of PGR, $H_2O_2$ and $Na_2B_4O_7$. The cobalt concentration was calculated by substituting the absorbance values, at a fixed time of 5 min, into the corresponding equation for the calibration graph. The results are shown in Table 2, the relative standard deviation ($s_r$) being in the range 15.9–0.7% for a cobalt concentration of 0.40–12.00 ng ml$^{-1}$.

The accuracy[19–22] was studied in the range 0.40–12.00 ng ml$^{-1}$ of Co. The homogeneity of the variances of the analysed samples was confirmed by application of Bartlett's test and Hartley's test. The linear regression of the values obtained for each analysis of each sample and the corresponding real values were obtained. The statistical $t$-test was applied to the study of the slope and the intercept of the straight line was obtained (Table 3). From this study we conclude that the proposed method does not present a constant-type error (a slope equal to unity) and it does not need a blank correction (an intercept equal to zero).

## Selectivity

The effect of metal ions on the cobalt-catalysed reaction is shown in Figs. 1 and 2, where $\Delta A$ is the difference in absorbance between the cobalt-catalysed reaction and the cobalt-catalysed - possible interferent species reaction, at a fixed time of 5 min.

From the results, the interfering processes may be classified as follows: (a) inhibition of the catalytic activity of cobalt in the presence of Cr(III), Ce(IV), Fe(III), Cu(II), Zn(II), Cd(II) and Cr(VI); (b) acceleration of the catalytic effect, in

**Table 3.** Statistical *t*-test applied to the study of the intercept and the slope

| Equation of straight line | $r$ | $s^2_{y,x}$ | *t*-Test (intercept) $s_a$ | *t*-Test (intercept) $t_{exp}$ | *t*-Test (slope) $s_b$ | *t*-Test (slope) $t_{exp}$ | $\nu = N-2$ | $\nu$ | $t_{1-\alpha/2} = t_{0.975}$ |
|---|---|---|---|---|---|---|---|---|---|
| $y = 0.04 + 0.997x$ | 0.9997 | 0.0488 | 0.042 | 1.074 | $6.84 \times 10^{-3}$ | −0.438 | 58 | 40 | 2.021 |
| | | | | | | | | 60 | 2.000 |

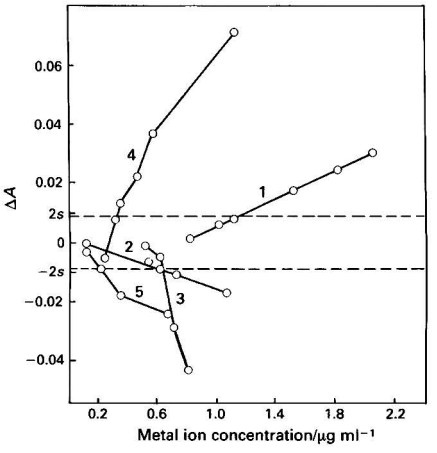


**Fig. 1.** Influence of metal ions on the kinetic - catalytic determination of cobalt. 1, Pb(II) 550; 2, Cr(III) 300; 3, Ce(IV) 300; 4, Mn(II) 150; and 5, Fe(III) 100 (the numbers given after each element refer to maximum tolerable ratios of foreign metal ion to analyte concentration). Conditions: PGR, $3.0 \times 10^{-5}$ M; $H_2O_2$, $3.5 \times 10^{-2}$ M; $Na_2B_4O_7$, $4 \times 10^{-2}$ M; Co, 2 ng ml$^{-1}$; pH, 8.3; $\lambda$ = 515 nm; and temperature, 25 °C

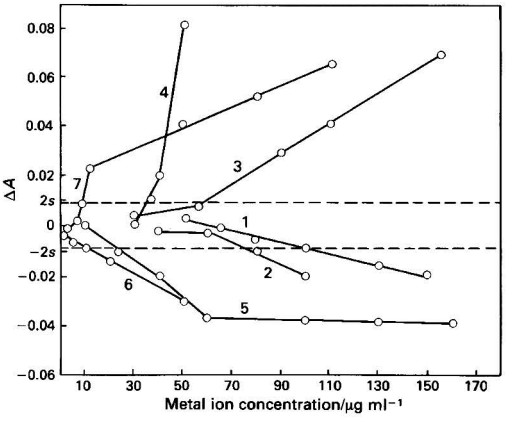


**Fig. 2.** Influence of metal ions on the kinetic - catalytic determination of cobalt. 1, Cu(II) 50; 2, Zn(II) 40; 3, Mn(VII) 28; 4, V(V) 18; 5, Cd(II) 12; 6, Cr(VI) 5; and 7, Ni(II) 4 (the numbers given after each element refer to maximum tolerable ratios of foreign metal ion to analyte concentration). Conditions: PGR, $3.0 \times 10^{-5}$ M; $H_2O_2$, $3.5 \times 10^{-2}$ M $Na_2B_4O_7$, $4 \times 10^{-2}$ M; Co, 2 ng ml$^{-1}$; pH, 8.3; $\lambda$ = 515 nm; and temperature, 25 °C

the presence of Pb(II), Mn(II), Mn(VII), V(V) and Ni(II); and (c) $10^4$ ng ml$^{-1}$ of $PO_4^{3-}$, $BrO_3^-$, of $ClO_4^-$, $ClO_3^-$, $IO_4^-$ and $IO_3^-$ do not interfere. A species is considered to cause interference when a change of more than twice the standard deviation of the absorbance value of the catalysed reaction ($s$ = $4.3 \times 10^{-3}$ absorbance units, calculated from 10 independent measurements with a cobalt concentration of 2 ng ml$^{-1}$) is obtained.

### Recommended Procedure

Samples containing between 5 and 325 ng of cobalt(II) were placed in 25-ml calibrated flasks, 10 ml of 0.1 M sodium tetraborate - 0.2 M hydrochloric acid buffer solution and 0.1 ml of 30% *m/V* hydrogen peroxide were added and the contents diluted to 20 ml with distilled water. This solution was shaken gently while 1 ml of $8 \times 10^{-4}$ M PGR was added. The stop-watch was turned on when the last drop had fallen and the solution was diluted to the mark with distilled water. The absorbance was measured at 515 nm at 25 °C against water as a reference blank, for a fixed time of 5 min. The cobalt concentration was calculated from the corresponding equation for the calibration graph.

## References


1. Otto, M., Muller, H., and Werner, G., *Talanta*, 1978, **25**, 123.
2. Chistyakov, N., *Sb. Nauchn, Tr. Ivanov. Gos. Med. Inst.*, 1965, **31**, 65.
3. Popa, Gr., and Costache, D., *Rev. Roum. Chim.*, 1967, **12**, 963.
4. Kucharkowski, R., and Döge, H. G., *Fresenius Z. Anal. Chem.*, 1968, **238**, 241.
5. Otto, M., Rentsch, J., and Werner, G., *Anal. Chim. Acta*, 1983, **147**, 267.
6. Costache, D., *Rev. Roum. Chim.*, 1970, **15**, 1181.
7. Prik, G. A., and Orlova, M. N., *Uch. Zap. Vladimir. Gos. Pedagog. Inst.*, 1970, **22**, 48.
8. Kreingol'd, S. U., Sosenkova, L. I., and Vzorova, I. F., *Metody Anal. Kontrol. Proizvod. Khim. Prom.*, 1976, No. 2, 38; *Anal. Abstr.*, 1977, **22**, 382.
9. Alexiev, A. A., and Angelova, M. G., *Mikrochim. Acta*, 1980, **II**, 187.
10. Blazys, I., Paeda, R., and Jurevicius, R., *Nauchn. Tr. Vyssh. Ucheb. Zaved. Lit. SSR, Khim. Khim. Tekhnol.*, 1971. **13**, 35.
11. Costache, D., and Junie, P., *Rev. Roum. Chim.*, 1971, **16**, 597.
12. Vershinin, V. I., Chuiko, V. T., and Reznik, B. E., *Zh. Anal. Khim.*, 1971, **26**, 1710.
13. Reznik, B. E., Chuiko, V. T., and Vershinin, V. I., *Zh. Anal. Khim.*, 1972, **27**, 395.
14. Popa, Gr., and Costache, D., *An. Univ. Bucuresti., Ser. Stiint. nat. Chim.*, 1968, **17**, 21.
15. Costache, D., and Popa, Gr., *Rev. Roum Chim.*, 1970, **15**, 1349.
16. Sevillano-Cabeza, A., Llobat-Estelles, M., and Medina-Escriche, J., *Analyst*, 1985, **110**, 1333.
17. IUPAC, "Compendium of Analytical Nomenclature," Pergamon Press, Oxford, 1978.
18. ACS Committee on Environmental Improvement, *Anal. Chem.*, 1980, **52**, 2242.
19. Mandel, J., and Linnig, F. J., *Anal. Chem.*, 1957, **29**, 743.
20. Commissariat à l'Energie Atomique, "Statistique Appliquée l'Exploitation de Mesures," Masson, Paris, 1978.
21. de la Guardia, M., Salvador, A., and Berenguer, V., *An. Quim.*, 1981, **77**, 129.
22. de la Guardia, M., Salvador, A., and Berenguer, V., paper presented at the 5° Encontro Anual da Sociedade Portuguesa da Química, 1982.


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# Effect of Anion-exchange Resin on the Formation of Iron(III) - Tiron Complexes

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A comparison between iron(III) - Tiron complexes in solution and in the presence of a finely divided anion-exchange resin suspension (ARS) was studied. The effects of the ARS on the acid dissociation constants of Tiron ($K_{a_1}$ and $K_{a_2}$) and on the stepwise stability constants ($K_1$, $K_2$ and $K_3$) of iron(III) - Tiron complexes (FeL, $FeL_2$ and $FeL_3$) were investigated. Instead of the three complexes formed in solution at different pH values, only two complexes ($FeL_2$ and $FeL_3$) were found to be fixed on the ARS. Although the ARS did not affect the acid dissociation constants of Tiron, the stability constants of $FeL_2$ and $FeL_3$ were considerably improved. The stepwise stability constants were 20.0, 18.5 and 15.0, respectively, compared with reported values in solution of 20.4, 15.1 and 10.8, respectively.

**Keywords:** *Iron(III) - Tiron complex formation; anion-exchange resin; stability constants*

The reactions of 1,2-dihydroxybenzene-3,5-disulphonate (Tiron) with metal ions are well documented and Tiron is well known as a colorimetric reagent for the determination of some metal ions.[1] Recently, molybdenum(VI) and vanadium(V) have been determined by densitometry after enrichment as the Tiron complex on a thin layer of anion-exchange resin.[2,3] This paper presents an experimental comparison between the metal - ligand system in solution and the metal - ligand system in the presence of a finely divided anion-exchange resin suspension (ARS). As the reaction between iron(III) and Tiron has been extensively studied, this system was selected in order to obtain a clearer insight into the effect of the anion-exchange resin on the complex formation between iron(III) and Tiron. This study was undertaken with the objective of determining the three stepwise stability constants of iron(III) - Tiron complexes in the presence of an ARS.

## Experimental

### Reagents

*Iron(III) standard solution*, $10^{-3}$ M. Prepared by dissolving 0.4822 g of ammonium iron(III) sulphate, $FeNH_4SO_4)_2.12H_2O$, in 100 ml of water containing 5 ml of sulphuric acid and diluting to 1 l.

*Iron(III) standard solution*, 1000 p.p.m. (1 mg $ml^{-1}$). Prepared by dissolving 8.6340 g of ammonium iron(III) sulphate in 100 ml of water containing 5 ml of sulphuric acid and diluting to 1 l. A working solution, containing 10 μg $ml^{-1}$, was prepared by appropriate dilution.

*Potassium hydrogen phthalate standard solution*, 0.1 M. Prepared by dissolving 5.1056 g of potassium hydrogen phthalate, after drying for 2 h at 120 °C, in boiled water. The solution was diluted to 250 ml.

*Sodium hydroxide standard solution*, 0.1 M. The solution was standardised against standard potassium hydrogen phthalate solution.

*Potassium chloride solution*, 1 M.

*Tiron solution*, 0.1 M. The reagent was obtained from Dojindo (Japan) and used without further purification. A 0.1 M aqueous solution was prepared and standardised against standard sodium hydroxide solution. Working solutions of $10^{-2}$ and $10^{-3}$ M were prepared by dilution.

*Buffer solutions*. The buffer solutions used for examining the effect of pH were 1 M sodium monochloroacetate - monochloroacetic acid, 1 M sodium acetate - acetic acid and 1 M ammonia - ammonium chloride solutions.

### Anion-exchange Resin

This was macroreticular Amberlyst A-27 (Rhom and Haas) in the chloride form. The anion-exchange resin suspension (ARS) with particles smaller than 30 μm was prepared according to the reported method.[4] The exchange capacity of the suspension, as determined by conductimetric titration, was 7.12 μequiv. $ml^{-1}$. A working suspension (3.56 μequiv. $ml^{-1}$) was prepared by dilution.

### Apparatus

A Shimadzu CS-920 Chromatoscanner was used for the measurement of the reflecting absorbance of the coloured complex in the thin layer of anion-exchange resin. A densitometer was used to linearise the convex calibration graph based on the Kubelka - Munk theory.[5,6] A Shimadzu Type UV-240 UV - visible recording spectrophotometer was used for measuring the absorption spectra of iron(III) - Tiron complexes in solution and in the resin phase. A Hiranuma Type RAT-11 recording autotitrator was used for carrying out the potentiometric titration of Tiron in the presence and absence of the ARS. A Hitachi - Horiba Type $F7_{LC}$ pH meter was applied for the adjustment of pH. A Toyo KG-25 filter holder was used for the preparation of the thin layer by filtration under suction. A TM-1 filter-paper (0.65 μm) (Toyo) was used.

### Determination of Stepwise Stability Constants

For the determination of $K_1$ and $K_2$, a 40-μl portion of $10^{-3}$ M iron(III) is placed in a dry 100-ml beaker followed by various amounts of $10^{-3}$ M Tiron solution. A 1.0-ml portion of acetate buffer solution and 2.0 ml of 1 M KCl solution are added. The sample volume is adjusted to 17.0 ml with water and then a 3.0-ml portion of ARS is added. The final pH of the solution is 3.67. The mixture is stirred for 10 min by means of a magnetic stirrer, then the resin is collected on a membrane filter under suction. A disc of coloured thin layer is formed that is 17 mm in diameter and about 0.2 mm in thickness. It is then wetted in a dipping solution containing $5 \times 10^{-2}$ M acetate buffer solution (pH 3.7). The wet membrane filter holding the resin is placed on a white plastic plate in the densitometer, then the integrated absorbance is measured using the lineariser at 600 nm by scanning the thin layer over an area 24 mm wide and 30 mm long. The blank value is obtained by carrrying out the procedure without the addition of iron(III).

The third stability constant, $K_3$, is determined in a similar manner except at pH 5.43 instead of pH 3.67.

As the violet thin layer has been found to change to red during storage in the dipping solution, it is necessary to

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measure the absorbance immediately after the preparation of the thin layer. All the experiments were carried out at room temperature (20 ± 1 °C).

## Results and Discussion

### Absorption Spectra

The absorption spectra of iron(III) - Tiron complexes were measured using the spectrophotometer for both the solution and the resin phase. The absorption spectra in solution were measured using a 1-cm cell against a reagent blank, whereas in the resin phase the thin layer of the sample and blank together were fixed on a glass plate fitted directly to the cell holder and the absorbance was measured with a slit width of 5 mm. Fig. 1 shows the absorption spectra of iron(III) - Tiron complexes in the solution. As expected,[1] the reagent produced three complexes with iron(III) ions depending on the pH of the solution; red ($FeL_3$) at pH higher than 7, violet ($FeL_2$) in slightly acidic solutions and blue (FeL) in more acidic solutions. The absorption maxima of each complex were 480, 560 and 670 nm, respectively. The absorption spectra of iron(III) - Tiron complexes in the resin phase are shown in Fig. 2. Only two complexes are found to be fixed on the resin phase: red ($FeL_3$) in basic and slightly acidic solutions (pH > 4.8) and violet ($FeL_2$) in more acidic solutions.

### Effect of Stirring Time

The influence of stirring time on the formation of the 1:2 complex was examined. The thin layers were prepared from solutions of pH 3.65, then wetted in a dipping solution containing $5 \times 10^{-2}$ M acetate buffer solution (pH 3.65). The absorbance measurements were carried out at 600 nm immediately after filtration. The absorbance was found to be constant up to 20 min, which indicates that the reaction between iron(III) and Tiron in the presence of the ARS is very fast and the reaction equilibrium can be attained quickly after the addition of the reagents.

### Effect of ARS on the Acid Dissociation Constants of Tiron

The influence of the ARS on the acid dissociation constants of Tiron was studied by potentiometric titration. A 50-ml portion of the test solution containing 5.0 ml of 0.1 M Tiron was titrated with 0.1 M standard sodium hydroxide solution in the absence and presence of 40.0 ml of ARS (3.56 μequiv. $ml^{-1}$). The ionic strength was kept constant (0.1). It was found that the titration curves in the presence and absence of the ARS coincide, which shows that the ARS has no effect on the acid dissociation constants of Tiron. The reported values of the acid dissociation constants of Tiron[1] were used in the calculation of the three stepwise stability constants of iron(III) - Tiron complexes.

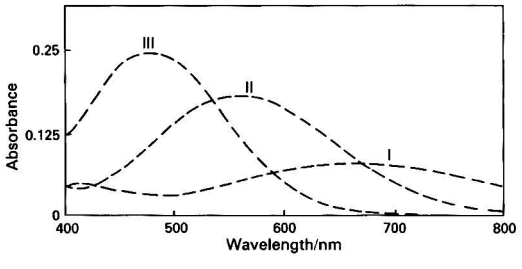


**Fig. 1.** Absorption spectra of iron(III) - Tiron complexes in solution. Iron(III), $4.0 \times 10^{-5}$ M; Tiron, $5.0 \times 10^{-3}$ M; KCl, 0.1 M. pH: I, 2.01; II, 4.95; and III, 7.70

### Calculation of $K_1$ and $K_2$

The total concentration of Tiron is given by

$$C_L = [L] + [HL] + [H_2L] + [FeL] + 2[FeL_2] + 3[FeL_3] \quad (1)$$

Introducing the acid dissociation constant of Tiron, the free ligand concentration is expressed by

$$[L] = \frac{C_L - ([FeL] + 2[FeL_2] + 3[FeL_3])}{1 + \frac{[H]}{K_{a2}} + \frac{[H]^2}{K_{a1}K_{a2}}} \quad \ldots (2)$$

where $K_{a1}$ and $K_{a2}$ are $10^{-7.66}$ and $10^{-12.6}$, respectively.[1] Although it has been found that almost all of the free Tiron is fixed on the resin phase, for simple calculation it is considered as if it is in the solution.

At pH 3.67, as the formation of $FeL_3$ is assumed to be negligible so the molar fraction of $FeL_2$ is given by

$$\phi_2 = \frac{[FeL_2]}{C_{Fe}} = \frac{K_1K_2[L]^2}{1 + K_h/[H] + K_1[L] + K_1K_2[L]^2}$$

where $C_{Fe}$ is the total concentration of iron(III), $K_1$ and $K_2$ are the stability constants of FeL and $FeL_2$ and $K_h$ is the hydrolysis constant of iron(III), reported as[7]

$$K_h = [FeOH][H]/[Fe] = 10^{-2.63} \quad (4)$$

As it was found that FeL could not be fixed on the resin phase, the colour of the thin layer is assumed to be due only to $FeL_2$. The absorbance of the thin layer may be proportional to the concentration of $FeL_2$ in the resin phase, that is,

$$A = k_{FeL_2}[FeL_2]_r \quad (5)$$

where $k_{FeL_2}$ is the proportionality constant and the subscript r denotes the resin phase. As the distribution coefficient of $FeL_2$ is given by

$$d_{FeL_2} = \frac{[FeL_2]_r}{[FeL_2]} \quad \ldots \quad \ldots \quad \ldots \quad (6)$$

the absorbance of the thin layer is proportional to the concentration of $FeL_2$ in the solution:

$$A = k_{FeL_2}\, d_{FeL_2}[FeL_2] \quad (7)$$

At the maximum colour formation the above equation can be written as

$$A_{max.} = k_{FeL_2}\, d_{FeL_2}[FeL_2]_{max.} \quad (8)$$

As shown in Fig. 3, the absorbance of the thin layer prepared from the solution containing 40 nmol of iron(III) increased with increasing amount of Tiron and reached a constant and

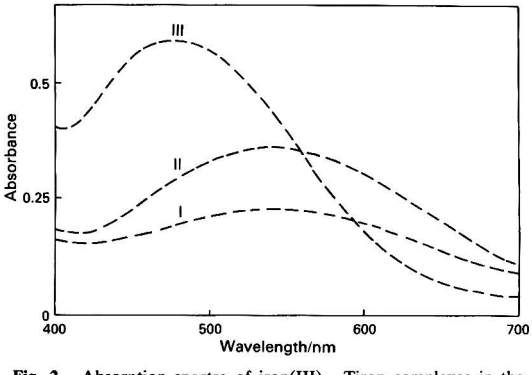


**Fig. 2.** Absorption spectra of iron(III) - Tiron complexes in the presence of the ARS. Iron(III), 2.0 μg; Tiron, $5.0 \times 10^{-4}$ M; buffer solution, $5 \times 10^{-2}$ M; KCl, 0.1 M; ARS (3.56 μequiv. $ml^{-1}$), 3.0 ml; sample volume, 20.0 ml. pH: I, 2.90; II, 3.67; and III, 5.43

maximum value. When the thin layer of maximum colour was wetted with the acetate buffer solution of pH 5.4, the colour changed as a result of the formation of $FeL_3$. The absorbance of the resulting thin layer was compared with that of the thin layer that had the full colour produced by 40 nmol of iron(III) as $FeL_3$. The value was found to be 0.97, which indicates that

$$\frac{[FeL_2]_{max.}}{C_{Fe}} = 0.97 \quad .. \quad .. \quad .. \quad (9)$$

From equations (3), (7), (8) and (9), we obtain

$$\phi_2 = 0.97A/A_{max.} \quad (10)$$

At pH 3.67, as the concentration of $FeL_3$ is assumed to be trivial, the concentration of free Tiron is given from equation (2) as

$$[L] = 10^{-12.92}\{C_L - ([FeL] + 2[FeL_2])\} \quad (11)$$

By introducing the numerical values of $K_h$ and pH into equation (3) and rearranging we obtain

$$\left(\frac{1}{\phi_2} - 1\right)[L]^2 = \frac{11.96}{K_1K_2} + \frac{1}{K_2}[L] \quad .. \quad (12)$$

If [L] is properly calculated from equation (11) and the left-hand side of equation (12) is plotted against [L], a straight line is obtained. The results are shown in Fig. 4. From the slope of the line and the intercept, $K_1$ and $K_2$ were obtained. The logarithmic values of the stability constants $K_1$ and $K_2$ were found to be 20.0 and 18.5, respectively.

### Determination of $K_3$

The experiments were carried out at pH 5.43, where the predominant species of iron(III) can be assumed to be $FeL_2$ and $FeL_3$. It can be predicted that with increasing amount of Tiron, first the concentration of $FeL_2$ will increase and the absorbance also increases, then with further addition of Tiron $FeL_3$ begins to be formed and the absorbance begins to decrease because the absorption coefficient of $FeL_3$ is lower than that of $FeL_2$ at 600 nm, as shown in Fig. 2. This prediction was supported by the results shown in Fig. 5. The absorbance was found to increase with increasing amount of Tiron; to reach a maximum and then to decrease to a constant value. The absorbance can be written as

$$A = k'_{FeL_2}d'_{FeL_2}[FeL_2] + k'_{FeL_3}d'_{FeL_3}[FeL_3] \; .. \quad (13)$$

where $k'$ and $d'$ denote the proportionality constant and the distribution coefficient at pH 5.43, respectively. If it is assumed that at the maximum absorbance total iron is present as $FeL_2$ and at the minimum absorbance it is present as $FeL_3$, then

$$A_{max} = k'_{FeL_2}d'_{FeL_2}C_{Fe} \quad (14)$$

and

$$A_{min} = k'_{FeL_3}d'_{FeL_3}C_{Fe} \quad (15)$$

When $[FeL_2] = [FeL_3] = C_{Fe}/2$, the absorbance is given by

$$A = (A_{max} + A_{min})/2 \quad (16)$$

and the corresponding concentration of free Tiron at pH 5.43 is given by equation (2) as

$$[L] = 10^{-9.40}\left(C_L - \frac{5}{2}C_{Fe}\right) \quad .. \quad .. \quad (17)$$

As $K_3 = [FeL_3]/[FeL_2][L]$, when the equation $[FeL_2] = [FeL_3]$ holds, the formation constant $K_3$ is given by

$$K_3 = 1/[L] \quad (18)$$

From the results shown in Fig. 5, one obtains $[L] = 10^{-15.0}$ and so the logarithmic value of the constant $K_3$ is found to be 15.0.

### Effect of pH on the Distribution of Iron(III) - Tiron Complexes at a Constant Tiron Concentration

The stability constants of iron(III) - Tiron complexes thus obtained in the presence of the ARS were $K_1 = 20.0$, $K_2 = 18.5$ and $K_3 = 15.0$ at an ionic strength of *ca.* 0.1, the reported values[1] in solution being 20.4, 15.1 and 10.8, respectively.

To obtain a clearer picture of the effect of the ARS on the iron(III) - Tiron complexes, the distribution of iron(III) between various complexes as a function of pH, at a constant Tiron concentration, was calculated. The distribution curves of iron(III) - Tiron complexes in solution are illustrated in Fig. 6(*a*) and in the presence of the ARS in Fig. 6(*b*). On comparing these curves, it is clear that the ARS has a marked effect on the distribution of the iron(III) - Tiron complexes. The range of pH at which FeL, $FeL_2$ and $FeL_3$ occur in the solution was shifted to lower values in the presence of the ARS. Fig. 6 also shows that the distribution percentage of FeL, which was found not to be fixed on the anion-exchange resin, decreased in the presence of the ARS. The influence of

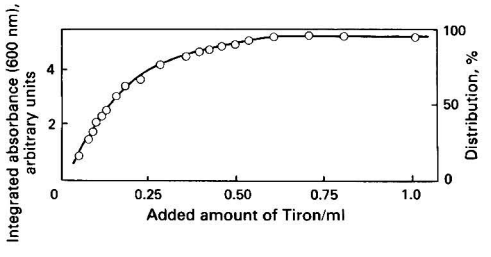


**Fig. 3.** Effect of amount of Tiron on the absorbance of the iron(III) - Tiron complex at pH 3.67. Iron(III), $2.0 \times 10^{-6}$ M; buffer solution, $5 \times 10^{-2}$ M; KCl, 0.1 M; ARS (3.56 μequiv. $ml^{-1}$), 3.0 ml; sample volume, 20.0 ml. The distribution percentage was calculated according to equation (10)

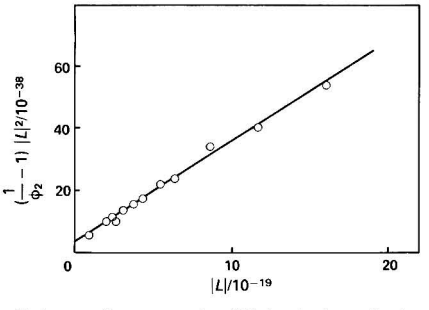


**Fig. 4.** Plots according to equation (12) for the determination of $K_1$ and $K_2$

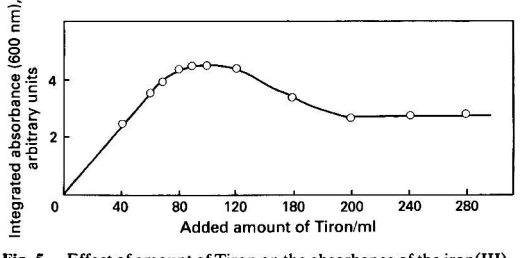


**Fig. 5.** Effect of amount of Tiron on the absorbance of the iron(III) - Tiron complex at pH 5.43. Iron(III), $2.0 \times 10^{-6}$ M; buffer solution, $5 \times 10^{-2}$ M; KCl, 0.1 M; ARS (3.56 μequiv. $ml^{-1}$), 3.0 ml; sample volume, 20.0 ml

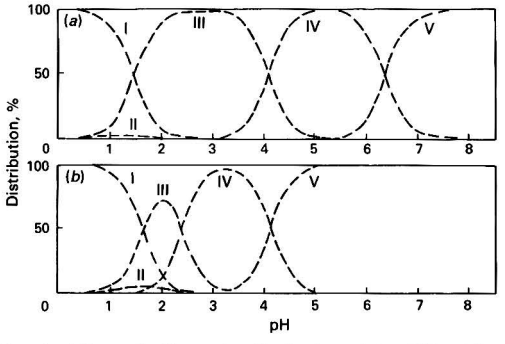


**Fig. 6.** Effect of pH on the distribution of iron(III) - Tiron complexes calculated at a constant Tiron concentration: [L] + [HL] + [$H_2L$] = $5.0 \times 10^{-4}$ M. (*a*) In solution; and (*b*) in the presence of the ARS. I, $Fe^{3+}$; II, FeOH; III, FeL; IV, $FeL_2$; and V, $FeL_3$

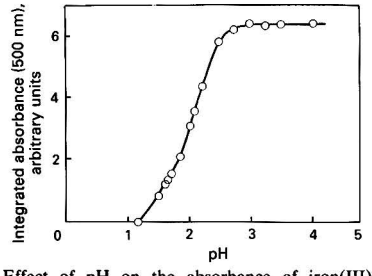


**Fig. 7.** Effect of pH on the absorbance of iron(III) - Tiron complexes. Iron(III), 2.0 μg; Tiron, $5.0 \times 10^{-4}$ M; buffer solution, $5 \times 10^{-2}$ M; KCl, 0.1 M; ARS (3.56 μequiv. $ml^{-1}$), 3.0 ml; sample volume, 20.0 ml

pH on the fixation of iron(III) - Tiron complexes was examined as shown in Fig. 7. The fixation of iron(III) as the Tiron complex on the ARS started at *ca.* pH 2 and the maximum fixation was observed at *ca.* pH 4, as predicted from the distribution curve of iron(III) - Tiron complexes (Fig. 6).

### Effect of Concentration of Tiron on the Distribution of Iron(III) - Tiron Complexes at a Constant pH

Fig. 8 shows the distribution of iron(III) between various complexes in the presence and absence of the ARS as a function of the concentration of free Tiron at pH 3.67. Fig. 8(*a*) shows the distribution of iron(III) - Tiron complexes in solution and Fig. 8(*b*) that in the presence of the ARS. Comparison of the two indicates that the presence of the ARS affects the distribution of the iron(III) - Tiron complexes. In the presence of the ARS a smaller amount of Tiron than that in solution was found to be sufficient for the formation of the complexes.

### Calibration Graph

The calibration graphs for the iron(III) - Tiron complex in the presence of the ARS were constructed at pH 4.8 and 9.0. On using the linearliser, good linearity was observed up to 3.0 μg of iron(III). It was also found that both lines had the same slope, which indicates that in the presence of the ARS the 1 : 3 complex is formed over a wide pH range.

### Effect of Potassium Chloride

The effect of potassium chloride on the fixation of the 1 : 2 and 1 : 3 iron(III) - Tiron complexes on the ARS was examined, as shown in Fig. 9. After fixation of iron(III) as $FeL_2$ at pH 3.67 on the ARS from the solution containing various amounts of

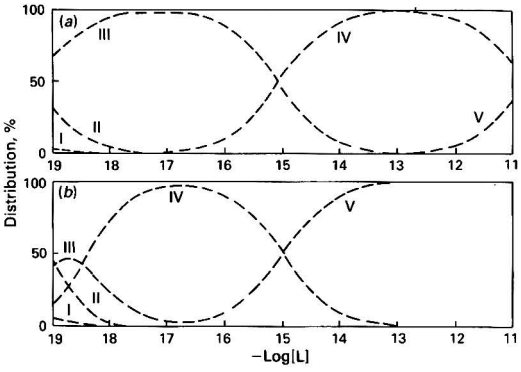


**Fig. 8.** Effect of Tiron concentration on the distribution of iron(III) - Tiron complexes calculated at a constant pH of 3.67. (*a*) In solution; and (*b*) in the presence of the ARS. I, $Fe^{3+}$; II, FeOH; III, FeL; IV, $FeL_2$; and V, $FeL_3$

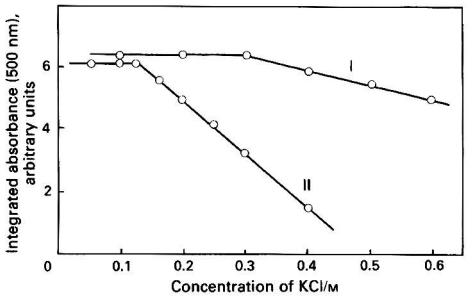


**Fig. 9.** Effect of potassium chloride on the fixation of $FeL_2$ and $FeL_3$ complexes. Iron(III), 2.0 μg; Tiron, $5.0 \times 10^{-4}$ M; buffer solution, $5 \times 10^{-2}$ M; ARS (3.56 μequiv. $ml^{-1}$), 3.0 ml; sample volume, 20.0 ml. I, $FeL_3$; and II, $FeL_2$

potassium chloride, the resulting thin layers of resin were wetted in the acetate buffer solution of pH 5.4 to convert $FeL_2$ in the resin phase into $FeL_3$, and then the absorbance was measured at 500 nm. The results were compared with those observed for $FeL_3$ at pH 5.43. It was found that the fixation of iron(III) as $FeL_2$ was constant up to 0.13 M of potassium chloride, then it started to decrease sharply with increasing concentration of the salt. On the other hand, the fixation of the 1 : 3 complex was constant up to 0.3 M of potassium chloride, then it started to decrease gradually with increasing concentration of the salt. It is clear that the 1 : 3 complex, having nine negative charges,[1] is more strongly fixed on the resin than the 1 : 2 complex, which has only five negative charges.

## References

1. Cheng, K. L., Ueno, K., and Imamura, T., "Handbook of Organic Analytical Reagents," CRC Press, Boca Raton, FL, 1982, pp. 63–69.
2. Shriadah, M. M. A., Kataoka, M., and Ohzeki, K., *Analyst*, 1985, **110**, 125.
3. Shriadah, M. M. A., and Ohzeki, K., *Analyst*, 1985, **110**, 677.
4. Abe, M., Ohzeki, K., and Kambara, T., *Bull. Chem. Soc. Jpn.*, 1978, **51**, 1090.
5. Yamamoto, H., Kurita, T., Suzuki, J., Hira, R., Nakano, K., and Makabe, H., *Bunseki Kagaku*, 1974, **23**, 1016.
6. Yamamoto, H., Kurita, T., Suzuki, J., Hira, R., Nakano, K., and Makabe, H., *J. Chromatogr.*, 1976, **116**, 29.
7. Sillen, L. G., and Martell, E. E., "Stability Constants of Metal Ion Complexes," Chemical Society, London, 1964, p. 54.

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# Multi-component Quantitative Analysis of Fluorescent Mixtures Not Obeying Beer's Law

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The determination of mixtures by a linear least-squares regression analysis is a well established technique. However, this mathematical approach depends on the individual components obeying the Beer - Lambert law. Although this assumption is generally true in UV absorption, the analysis of mixtures by fluorescence spectrometry presents a number of problems. Distortion of the sample spectra can occur through a variety of reasons, including energy transfer. By using standards that are mixtures of the components, rather than pure components, compensation can be made for non-linear deviations.

**Keywords:** *Least-squares multi-component analysis; fluorimetry; non-linear deviation*

Multi-component analysis using regression techniques has received much attention during recent years. The growth in the use of the technique has been stimulated by new instrument designs using microprocessors and the advent of powerful desk-top computers using advanced software routines. For the quantitative analysis of multi-component mixtures the most popular mathematical technique is that of least squares using standard matrix algebra.[1–4] However, an assumption is made that the absorbance (or fluorescence) response is a function of the known components and that they obey the Beer - Lambert law. Several attempts have been made to accommodate these difficulties. Leggett[5] and Warner *et al.*[6] proposed the use of non-negative least squares to overcome the problem of negative molar absorptivities. Haaland and Easterling[7] and Brown *et al.*[8] selected only the spectral regions that gave the best agreement to the calibration spectra. Over-determination of the regression by using more standards than there are components was found by Maris *et al.*[9] to improve greatly the accuracy of most analyses. Osten and Kowalski[10] used a modification of self-modelling curve resolution as a means of testing and compensating for a background interferent.

Least-squares curve fitting involves finding that combination of a set of standard spectra which gives the best fit to a sample spectrum according to a least-squares criterion. These standard spectra have usually been those of the pure components, or spectra of the individual components derived from known mixtures. The calculated concentrations in the samples are then proportional to the contributions of these standard spectra to the best fit combination. An alternative is to use as the standards spectra from mixtures of known composition. The calculated concentration for each component is found by adding the contributions for that component from each standard.

The approach has been successfully applied to the determination of salicylic acid in aspirin.[11] The low levels of salicylic acid present as a contaminant can easily be calculated even though very small differences occur between the fluorescence emission of acetylsalicylic acid and salicylic acid.

An advantage of this method is that it allows a new approach to systems showing deviations from the Beer-Lambert law. The spectrum of such a system can be represented as the sum of the spectra of the pure components, with appropriate weightings, plus an additional spectral component. We can define the additional spectral component as the difference between the observed spectrum of the mixture and the spectrum calculated from the spectra of the pure components on the basis of the Beer - Lambert law. Previous workers[12,13] have chosen as the additional spectral component the difference between the observed spectrum and the least-squares fit spectrum obtained from pure component spectra. Neither choice has any special physical significance. By using in the curve fitting procedure a number of standard spectra exceeding the number of known constituents in the sample, it is possible to take account of such additional spectral components.

Accurate quantitative measurements for the constituents can be obtained in this way without any knowledge of the form or concentration dependence of the additional spectral components.[14] The only requirements are that the additional spectral components should not simply be linear combinations of the spectra of the known constituents and that the spectra of the mixtures used as standards should include these additional components.

In this paper we shall show that this technique can be applied to samples whose emission spectra are distorted through energy transfer. Energy may be transferred from one component A (the donor), to another B (the acceptor), when the emission of A overlaps the absorption band of B. The reduction in the emission of A will generally not be the same for all wavelengths, but will depend on the absorption spectrum of B. The emission spectra will therefore not be a linear combination of the emission spectra of A and B with weightings proportional to their concentrations. It is possible to consider the deviations from linear behaviour as arising from an additional spectral component that is a combination of the emission of A and the absorption and emission of B, assuming that a constant proportion of the transferred energy is re-emitted by B. The example chosen is the energy transfer shown to occur between $Tb^{3+}$ chelated to dipicolinic acid, $Tb(DPA)_3$, and Rhodamine B.[15] Both the absorbance and fluorescence emission were directly proportional to their concentrations in the absence of each other. Quantitative analysis of mixtures by curve fitting using pure standards results in large errors. However, by using an additional standard, which is a mixture, there is an enormous improvement in accuracy.

## Experimental

### Instrumentation and Software

A Perkin-Elmer Model LS-5 luminescence spectrometer fitted with a red-sensitive R 928 photomultiplier and equipped with an RS 232C serial interface was used for time-resolved measurements and all luminescence excitation and emission studies. Data were recorded using a Perkin-Elmer Model 7500

Professional computer using the PECLS-III applications software providing instrumental control, data manipulation and storage. Quantitative analyses were performed using the QUANT-III software, which can use up to 15 standard spectra to match the spectrum of the sample.

### Reagents

All experiments were performed with analytical-reagent grade chemicals, except where stated otherwise. Water was distilled and passed through a 0.45-μm Millipore filter. Terbium(III) chloride ($TbCl_3.6H_2O$), 99.9%, was obtained from Aldrich, Gillingham, UK, dipicolinic acid from Fluka, Buchs, Switzerland, Rhodamine B from BDH Chemicals, Poole, UK, and Tris buffer (pH 8.0) from Sigma, Poole, UK.

### Procedure

All reagents and experimental solutions were prepared as described by Thomas *et al.*[15] Time-resolved fluorescence measurements were made using the Model LS-5 as described previously.[16] Fluorescence spectra were recorded with a delay time, $t_d$, of 0.01 ms and a gate time, $t_g$, of 8.0 ms. Emission spectra were recorded with a 5-nm spectral band pass and were corrected for instrumental response from 250 to 700 nm.

## Results and Discussion

The excited-state lifetime of $Tb(DPA)_3$ in the absence of Rhodamine B was found to be 2.08 ms, with its emission spectrum overlapping the absorption spectrum of Rhodamine B (Fig. 1). A series of mixtures were prepared containing terbium varying in concentration between 1.25 and 10.0 μM. The limit of 0.5 μM for the Rhodamine B concentration was chosen so as to keep the absorbance at 554 nm below 0.05 and to minimise the "inner filter" effect. A gate time of 8.0 ms was chosen so as to integrate the total emission from the terbium excited state.

The effect of energy transfer on the emission spectrum of the sample mixture is shown in Fig. 2. Spectrum B is the actual emission of the mixture and spectrum A is the result of adding the two components mathematically. There is an increase in the Rhodamine B emission while at the same time there is a decrease in the terbium emission. The excited-state lifetime of the $Tb(DPA)_3$ also decreased from 2.08 to 0.43 ms in the presence of 2 μM Rhodamine B. The latter also exhibited an emission lifetime virtually comparable to that of the $Tb(DPA)_3$, indicating "triplet" to singlet energy transfer. The emission spectra were measured and quantitatively analysed using the QUANT-III software using either two pure standards or with an additional standard that was a known mixture. The results are given in Table 1.

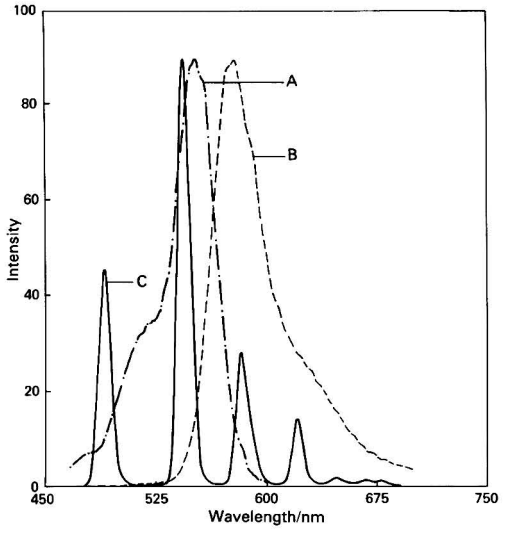


**Fig. 1.** (A) The corrected excitation and (B) the emission of Rhodamine B and (C) the corrected emission of $Tb(DPA)_3$, all measured with a 5-nm spectral band pass at an excitation of 260 nm

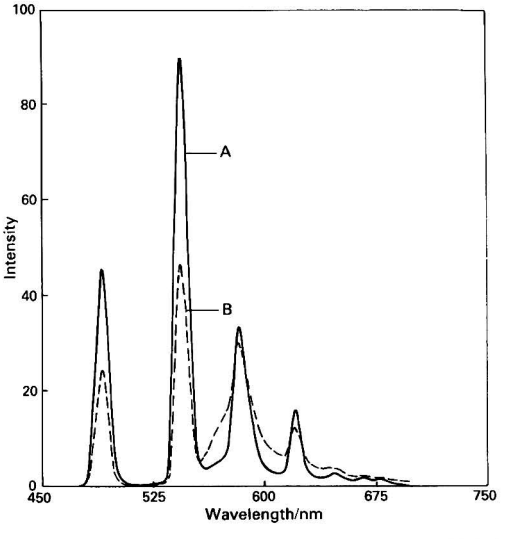


**Fig. 2.** (A) Synthetic spectrum of the two components and (B) the corrected emission of a mixture containing 0.5 μM Rhodamine B and 5.0 μM $Tb^{3+}$

**Table 1.** Analysis of $Tb(DPA)_3$ - Rhodamine B solutions

| Initial concentrations/μM | | Calculated/μM | | | |
|---|---|---|---|---|---|
| | | A* | | B† | |
| $Tb(DPA)_3$ | Rhodamine B | $Tb(DPA)_3$ | Rhodamine B | $Tb(DPA)_3$ | Rhodamine B |
| 10.0 | 0.5 | 5.25 | 2.20 | 10.26 | 0.48 |
| 5.0 | 0.5 | 2.66 | 1.25 | 4.86 | 0.47 |
| 2.5 | 0.5 | 1.25 | 0.91 | 2.50 | 0.49 |
| 1.25 | 0.5 | 0.64 | 0.78 | 1.35 | 0.50 |
| 2.5 | 0.25 | 1.77 | 0.53 | 2.51 | 0.27 |
| 1.25 | 0.125 | 1.02 | 0.20 | 1.24 | 0.131 |

* A, Two standards, 2.5 μM $Tb(DPA)_3$ and 1.0 μM Rhodamine B.
† B, Three standards, as A plus 5.0 μM $Tb(DPA)_3$, 0.5 μM Rhodamine B.

Quantitative analysis using the spectra of the separate components results in gross errors in the relative concentrations. With a single additional standard that is a mixture there is a considerable improvement in accuracy, the errors being reduced on average by a factor of 20.

By specifying that the mixture contains three components and setting the nominal concentrations of the three standards used in analysis B to 1.0, an estimate can be obtained of the contribution due to energy transfer. Normalising these values to the terbium concentration and plotting the results against the corresponding Rhodamine B concentration yields a linear relationship, indicating that the transferred energy is proportional to the Rhodamine B concentration up to 0.5 μM. Peak-area calculations on spectra for actual and synthetic mixtures indicate that there is an approximate 10% loss in the amount of energy transferred.

## Conclusion

Accurate quantitative analysis of fluorescent mixtures using least-squares curve fitting can be achieved even when there are gross deviations from the Beer - Lambert law. By including a standard that is a known mixture, the errors that occur when separate components are used can be considerably reduced.

## References

1. Sternberg, J. C., Stillo, H. S., and Schwendmeman, R. H., *Anal. Chem.*, 1960, **32**, 84.
2. Weber, G., *Nature (London)*, 1961, **190**, 27.
3. Ainsworth, S., *J. Phys. Chem.*, 1963, **67**, 1613.
4. Meites, L., *Anal. Chim. Acta*, 1975, **74**, 177.
5. Leggett, D. J., *Anal. Chem.*, 1977, **49**, 276.
6. Warner, I. M., Davidson, E. R., and Christian, G. D., *Anal. Chem.*, 1977, **49**, 2155.
7. Haaland, D. M., and Easterling, R. G., *Appl. Spectrosc.*, 1982, **36**, 665.
8. Brown, C. B., Lynch, P. F., Obremski, R. J., and Lavery, D. S., *Anal. Chem.*, 1982, **54**, 1472.
9. Maris, M. A., Brown, C. W., and Lavery, D. S., *Anal. Chem.*, 1983, **55**, 1694.
10. Osten, D. W., and Kowalski, B. P., *Anal. Chem.*, 1985, **57**, 908.
11. Winfield, S. A., and Rhys Williams, A. T., *J. Pharm. Biomed. Anal.*, 1984, **2**, 561.
12. Antoon, M. K., Koenig, J. H., and Koenig, J. L., *Appl. Spectrosc.*, 1977, **31**, 518.
13. Ramana Rao, G., and Zerbi, G., *Appl. Spectrosc.*, 1984, **38**, 795.
14. Ford, M. A., and Spragg, R. A., *in* "Proceedings of the 1985 International Conference on Fourier and Computerised Infrared Spectroscopy, Ottawa," SPIE, USA, to be published.
15. Thomas, D. D., Carlsen, W. F., and Stryer, L., *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 5746.
16. Rhys Williams, A. T., and Fuller, M. J., *Computer Enhanced Spectrosc.*, 1983, **1**, 145.

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# Minimisation of Bilirubin Interference in the Determination of Fluorescein Using First-derivative Synchronous Excitation Fluorescence Spectroscopy

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The use of first-derivative synchronous excitation fluorescence is described for the minimisation of bilirubin interference in the determination of fluorescein. The limits of detection and the standard errors of estimates are better using the synchronous first-derivative approach than with conventional subtraction of the bilirubin blank. Results are shown for the quantitation of fluorescein in the nanomole concentration range.

**Keywords:** *Fluorescein determination; first-derivative synchronous excitation fluorescence; interferent elimination; bilirubin interference*

The use of fluorescein–conjugated reagents has become widespread, especially for use in fluorescence immunoassay techniques. Many of the immunoassays are applied to the analysis of biological fluids, especially serum.

However, the fluorimetric determination of fluorescein-labelled species in serum is subject to interference from high concentrations of serum bilirubin, such as in icteric sera of jaundiced patients.[1,2] The interference is due to the broad fluorescence emission and excitation bands of bilirubin that entirely overlap those of fluorescein. Free bilirubin is very unstable in aqueous media and has a negligible quantum yield ($10^{-5}$).[3] Bilirubin will, however, strongly bind to serum albumin, resulting in an increased quantum yield of 0.001.[4] The extent of interference is determined by the relative fluorescence intensity contributions of fluorescein (F) and bilirubin (B) and, assuming a quantum yield of 0.90 for fluorescein,[5] the relative bilirubin contribution will be 1% or more of the total fluorescence when $C_B \geq 30C_F$ (where $C$ is the concentration).

Bilirubin interference is generally treated by simple subtraction of the serum sample blank fluorescence from the total fluorescence signal (fluorescein + blank). The limits of detection (LOD) for the determination of fluorescein will then be limited by the relative magnitudes of the blank and the fluorescein contributions. At very high values of $C_B/C_F$ this difference will be small relative to the signals causing an increase in the LOD.

The coupling of synchronous excitation luminescence with derivative spectroscopy has been discussed previously.[6] For example, second-derivative synchronous excitation luminescence was used for the analysis of mixtures of acenaphthene, biphenyl, chrysene, dibenzothiophene and phenol.[7] The technique has also been used for the determination of cadmium using benzyl 2-pyridyl ketone 2-quinolylhydrazone.[8] The use of first-derivative synchronous excitation fluorescence spectroscopy is described for the selective determination of fluorescein in the presence of bilirubin. This approach offers a lower LOD and a wider range of linearity than is achieved using blank subtraction.

## Theory

The theory and applications of synchronous excitation fluorescence spectroscopy have been described in detail elsewhere,[9–13] as have those for derivative spectroscopy.[14,15] Briefly, in synchronous excitation fluorescence the excitation and emission monochromators are scanned simultaneously, maintaining a constant wavelength difference ($\Delta\lambda$) between the two monochromators. The $\Delta\lambda$ is usually chosen to equal the difference between the 0–0 transitions for fluorescence excitation and emission. The result of synchronous excitation fluorescence scanning is a narrowing of the spectral bands owing to the synchronous multiplication of the simultaneously increasing and then decreasing fluorescence spectra as functions of excitation and emission wavelengths. The synchronous excitation fluorescence intensity ($I_F$) takes the form

$$I_F(\lambda_{ex.},\lambda_{em.}) = kc[\mathrm{Ex}(\lambda_{ex.}) \times \mathrm{Em}(\lambda_{ex.} + \Delta\lambda) \ldots \quad (1)$$

where $c$ is the analytical concentration of the analyte; $k$ is a constant that contains various parameters (molar absorptivity, cell path length and instrumental parameters); $\lambda_{ex.}$ and $\lambda_{em.}$ are the excitation and emission wavelengths, respectively; and Ex and Em refer to the emission and excitation spectra, respectively.

The coupling of synchronous luminescence with derivative techniques simply involves taking the derivative of the synchronous spectrum. This can be carried out in real time or after spectral acquisition.[16]

## Experimental

### Materials

Bilirubin was purchased from United States Biochemical Co. (Cat. No. 12110) and used without further purification. Human serum albumin was purchased from Sigma (HSA, Cat. No. A1887). A stock solution of 106 μM HSA was prepared by dissolving 0.7000 g of HSA in pH 7.00, 0.10 M phosphate buffer and diluting to 1.000 dl followed by sonication for 30 min. This solution was subsequently diluted 10-fold with the buffer to yield a 10.6 μM HSA working solution. A stock solution of 1.00 mM bilirubin was prepared in 1.00 mM HSA by dissolving 0.0059 g of bilirubin and 0.6700 g of HSA in the buffer and diluting to 0.100 dl followed by sonication, in the dark, for 30 min. Laboratory standards (Set 1) of bilirubin were prepared in 1.00 mM HSA by adding the appropriate volume of stock solution followed by dilution to 1.000 ml. A second series of bilirubin standards (Set 2) were purchased from Sigma (Cat. No. 550-11) and contained 2.2, 9.9 and 15.0 mg $dl^{-1}$ of bilirubin. Fluorescein was purchased as the sodium salt from Sigma (Cat. No. F-6377) and a 1.00 mM stock solution was prepared by dissolving 0.0367 g of fluorescein and diluting to 1.000 dl with buffer solution followed by sonication for 30 min. This stock solution was subsequently diluted 10 000-fold with 10.6 μM HSA. All bilirubin solutions were kept in the dark to minimise potential sample degradation.

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All synchronous fluorescence excitation measurements were made using disposable polyethylene cuvettes (NSG Precision Cells, Inc.).

### Data Collection

Synchronous fluorescence spectra were collected on an SLM 4800S (SLM-Aminco) spectrofluorimeter with a 450-W Xe arc source and PMT detection. All spectra were collected in the "10 average" mode in which an average value is obtained via integration and division over approximately 3 s. The spectrofluorimeter was interfaced to an Apple II+ microcomputer for spectral acquisition and subsequent calculation of the first-derivative spectra. Measurements were taken in a ratiometric mode to minimise effects from source output fluctuations. Fifteen synchronous spectra were recorded for each sample and blank solution and the average of the 15 spectra was used for the calculation of the derivative spectrum.

The sample-chamber temperature was maintained at 20.0 ± 0.1 °C using a Haake A81 temperature control unit.

Monochromators were synchronously scanned from $\lambda_{ex.}$ = 440 nm to 540 nm at 0.5-nm intervals (200 points) maintaining a $\Delta\lambda$ of 20 nm between the excitation and emission monochromators and requiring approximately 10 min per spectrum. All monochromator entrance and exit slits and the modulation tank chamber exit slit were set at 16 nm.

### Data Analysis

The synchronous fluorescence excitation spectra were analysed by both blank subtraction and the first-derivative synchronous fluorescence excitation approaches. For the blank subtraction approach the synchronous spectra for fluorescein, bilirubin and mixtures of bilirubin and fluorescein were collected and the appropriate spectrum of bilirubin was subtracted from the spectrum of a mixture containing the same bilirubin concentration. The difference spectrum, corresponding to the fluorescein contribution, was used to determine the concentration of fluorescein from a calibration graph generated from fluorescein standards. In the first-derivative synchronous excitation fluorescence approach, the first-derivative synchronous excitation spectra were numerically generated by the Apple II+ and the differential intensities ($dF/d\lambda$) for the mixtures were measured at the point at which the bilirubin first-derivative synchronous spectrum gave zero contribution (see below). Fluorescein concentrations were determined using a calibration graph generated with measurements of standard first-derivative synchronous spectra at the same zero bilirubin point.

## Results and Discussion

The synchronous fluorescence spectra acquired at $\Delta\lambda$ = 20 nm for bilirubin, fluorescein and a mixture of the two species are shown in Fig. 1. Other $\Delta\lambda$ values were also used, but the best results were achieved using 20 nm, which is very close to the 0–0 transition for fluorescein (30 nm). The bilirubin fluorescence spectrum began to broaden significantly above the 20-nm value. It is evident that one cannot determine fluorescein devoid of bilirubin contribution at any of the wavelengths shown in Fig. 1.

The first derivatives of the synchronous spectra are shown in Fig. 2. The effects of the first-derivative transformation are dramatic and one feature is of special interest. The zero crossing point for bilirubin (481/501 nm) is very nearly at the maximum for the first-derivative spectrum of fluorescein, which is the optimum situation for fluorescein determination.

Table 1 shows the composition of the fluorescein and bilirubin standards used in this study. Fifty-four mixtures were

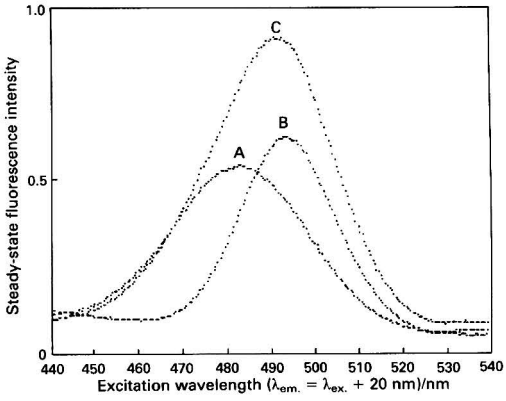


**Fig. 1.** Synchronous fluorescence spectra ($\Delta\lambda$ = 20 nm) for: A, 2.00 μM of bilirubin; B, 10 nM of fluorescein; and C, a mixture of 2.00 μM of bilirubin and 10 nM of fluorescein, all in 10 μM HSA

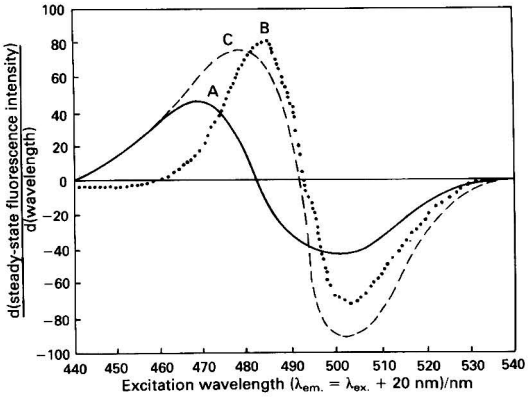


**Fig. 2.** First derivatives of the synchronous spectra shown in Fig. 1: A, bilirubin; B, fluorescein; and C, mixture

**Table 1.** Concentrations of standard solutions used in this study*

| | Bilirubin/μM | |
|---|---|---|
| Fluorescein/nM | Set 1† | Set 2‡ |
| 0.100 | 0.0977 | 3.67 |
| 0.200 | 0.244 | 1.65 |
| 0.398 | 0.489 | 2.51 |
| 0.990 | 0.977 | |
| 1.96 | 1.47 | |
| 3.23 | 1.95 | |

* Analytical concentration in cuvettes containing 10.6 μM HSA.
† Standards prepared from solid bilirubin (USB).
‡ Standards from Sigma.

used for the fluorescein determinations, prepared using each fluorescein standard with every bilirubin standard listed in Table 1. The LOD, which is the concentration of fluorescein ($C_F$) required to give a signal equal to the following:

$$C_F = \bar{x}_{ave.,blank} + 3S_{blank} \quad \ldots \quad (2)$$

where $\bar{x}_{ave.,blank}$ is the average value of the bilirubin blank signal and $S_{blank}$ is the standard deviation of $\bar{x}_{ave.,blank}$, determined as a function of bilirubin concentration at three wavelength pairs (481/501, 495/515 and 500/520 nm). The

**Table 2.** LOD results for fluorescein using 15 synchronous excitation spectra

| | LOD/nM | | | |
|---|---|---|---|---|
| | Blank subtraction (non-derivative) | | | |
| Bilirubin/mol l$^{-1}$ | 481/501 nm* | 495/515 nm* | 500/520 nm* | First derivative, 481/501 nm* |
| $9.77 \times 10^{-8}$† | 0.644 | 0.461 | 0.439 | 0.250 |
| $2.44 \times 10^{-7}$† | 0.896 | 0.531 | 0.533 | 0.360 |
| $4.89 \times 10^{-7}$† | 1.22 | 0.781 | 0.804 | 0.520 |
| $9.77 \times 10^{-7}$† | 2.07 | 1.42 | 1.56 | 1.04 |
| $1.47 \times 10^{-6}$† | 2.54 | 1.97 | 2.00 | 1.42 |
| $1.95 \times 10^{-6}$† | 2.99 | 2.21 | 2.54 | 1.66 |
| $3.67 \times 10^{-6}$‡ | 1.02 | 0.694 | 0.661 | 0.436 |
| $1.65 \times 10^{-6}$‡ | 2.65 | 2.04 | 2.15 | 1.53 |
| $2.51 \times 10^{-6}$‡ | 3.25 | 2.57 | 2.90 | 1.80 |
| $r$§ | 0.9987 | 0.9981 | 0.9977 | 0.9973 |
| SEE¶ | 0.059 | 0.058 | 0.072 | 0.052 |

*Excitation/emission wavelength pairs.
† Standards prepared in our laboratory (USB).
‡ Standards from Sigma.
§ Correlation coefficients for LOD *versus* bilirubin plots (second-order polynomial fit).
¶ Standard error of estimate (nM).

standard deviation for the bilirubin blank increases as the bilirubin concentration increases, resulting in an increase in the LOD for the determination of fluorescein using blank subtraction.

For the first-derivative work the $dF/d\lambda$ at the 481/501 nm wavelength pair was used and the fluorescein concentration determined from a calibration graph determined under the same set of conditions using the standards described in Table 1. Blank subtraction was not necessary as bilirubin does not contribute at the 481/501 nm wavelength pair under the first-derivative conditions. The limit of detection was also determined for this approach as a function of bilirubin concentration using the standard deviation for the blanks (bilirubin) at the various bilirubin concentrations (Table 1).

Table 2 shows the LOD results obtained by the blank subtraction (non-derivative) method at the 481/501, 495/515 and 500/520 nm wavelength pairs and those obtained by the first-derivative method at the 481/501 nm wavelength pair. The first-derivative approach gives significantly lower detection limits for the determination of fluorescein in the presence of bilirubin, owing to the minimisation of bilirubin contribution and therefore the imprecision associated with its contribution under the first-derivative conditions. Table 2 also lists the standard errors of estimates (SEE) under the various conditions, which are a measure of the precision for a given determination method. The first-derivative synchronous excitation approach is again shown to be the better method, and would be worth the additional time required for the acquisition of the synchronous spectrum in samples for which the minimisation of detection limits is of critical importance. The spectral acquisition time could be reduced to a negligible amount if a multi-channel detector was used.

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## References

1. Chiamori, N., Henry, R. J., and Golub, O. J., *Clin. Chim. Acta*, 1961, **8**, 1.
2. Bright, F. V., and McGown, L. B., *Anal. Chim. Acta*, 1984, **162**, 275.
3. Smith, D. S., Hassan, M., and Nargessi, R. D., *in* Wehry, E. L., *Editor*, "Modern Fluorescence Spectroscopy," Volume 3, Plenum Press, New York, 1981, Chapter 4.
4. Chen, R. F., *Biochem. Biophys.*, 1974, **160**, 106.
5. Weber, G., and Teale, F. W. J., *Trans. Faraday Soc.*, 1957, **53**, 646.
6. John, P., and Soutar, I., *Anal. Chem.*, 1976, **48**, 520.
7. Sanchez, F. G., Navas, A., and Santiago, M., *Anal. Chim. Acta*, 1985, **167**, 217.
8. Vo-Dinh, T., *in* Wehry, E. L. *Editor*, "Modern Fluorescence Spectroscopy," Volume 4, Plenum Press, New York, 1981, Chapter 5.
9. Vo-Dinh, T., Gammage, R. B., Hawthorne, A. R., and Thorgate, J. H., *Environ. Sci. Technol.*, 1978, **12**, 1297.
10. Lloyd, J. B. F., and Evett, I. W., *Anal. Chem.*, 1977, **49**, 1711.
11. Lloyd, J. B. F., *Analyst*, 1975, **100**, 82.
12. Vo-Dinh, T., *Anal. Chem.*, 1978, **50**, 396.
13. Vo-Dinh, T., and Gammage, R. B., *Anal. Chem.*, 1978, **50**, 2054.
14. Green, G. L., and O'Haver, T. C., *Anal. Chem.*, 1974, **46**, 2191.
15. O'Haver, T. C., *Anal. Chem.*, 1979, **51**, 91A.
16. O'Haver, T. C., and Green, G. L., *Anal. Chem.*, 1976, **48**, 312.

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# Novel Aryl Oxalate Esters for Peroxyoxalate Chemiluminescence Reactions

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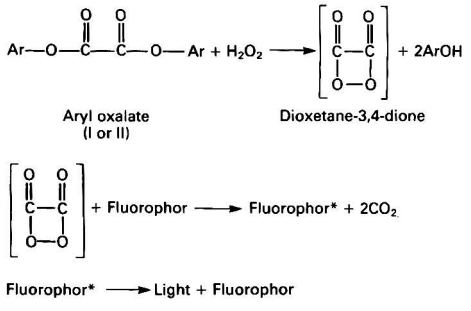
Six oxalate esters of 2-nitro-4-alkoxycarbonylphenol and 2-alkoxycarbonyl-4-nitrophenol were synthesised for use in the peroxyoxalate chemiluminescence reactions that take place via several steps with 4-hydroxy-3-nitrobenzoic acid and 5-nitrosalicylic acid as starting materials, respectively. The addition of ethylene glycol to the alkoxy moiety enhanced the solubility in solvents such as acetone, acetonitrile and ethyl acetate. When reacted with hydrogen peroxide in the presence of a fluorescent compound, perylene, they gave similar chemiluminescence reaction curves to those of DNPO [bis(2,4-dinitrophenyl) oxalate] and TCPO [bis(2,4,6-trichlorophenyl) oxalate]. Among them, bis[4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl] oxalate was found to be the best for the high-sensitivity detection of both hydrogen peroxide and fluorescent compounds using the peroxyoxalate chemiluminescence reaction because of its extreme solubility in solvents such as acetone (767 mM) and acetonitrile (1010 mM).

**Keywords:** *Aryl oxalates; bis[4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl] oxalate; chemiluminescence; fluorescent compounds; hydrogen peroxide*

Aryl oxalate esters and hydrogen peroxide generate chemiluminescence[1–3] in the presence of fluorescent compounds. This reaction (shown in Scheme 1) can be used for the detection of hydrogen peroxide produced in enzymic reactions[4,5] or enzyme immunoreactions.[6] It has also been applied successfully to the detection of fluorescent compounds on a thin-layer plate[7] and in high-performance liquid chromatography (HPLC).[8–15] In HPLC, its application to post-column reactions produced a sensitivity for the detection of the fluorescent compounds one to two orders of magnitude higher than did conventional light-induced fluorimetry; dansylated amino acids,[8,9] dansylated steroids,[10] fluorescamine-labelled catecholamines,[11] *o*-phthalaldehyde (OPA)-derivatised and nitrobenzofurazan (NBD)-labelled amines[12] and polycyclic aromatic hydrocarbons[13–15] were detected at the femtomole level.




**Scheme 1.** Proposed mechanism for the peroxyoxalate chemiluminescence reaction

For more sensitive detection using this reaction system, esters should be present in the reaction medium in larger amounts as the proposed active intermediate, dioxetane-1,2-dione, which excites the fluorescent compounds, can best be produced in the presence of large amounts of esters. However, bis(2,4,6-trichlorophenyl) oxalate (TCPO) and bis(2,4-dinitrophenyl) oxalate (DNPO), which are commonly used in the detection system for HPLC,[8–16] dissolve in acetone and acetonitrile to give about 10 mM solutions, which might be suitable solvents for the post-column reaction in HPLC.[8]

Therefore, we studied various oxalate esters that are more soluble than TCPO and DNPO in hydrophilic solvents. We selected as parent compounds 4-hydroxy(or 2-hydroxy)-3-nitro(or 5-nitro)benzoic acid (**III** or **IV**) (Fig. 1), as the nitro group in the *ortho*- or *para*-position to the hydroxy group greatly withdraws the electrons to yield a higher reactivity. The other reason is that the nitro group does not produce quenching like the chloro moiety of 2,4,6-trichlorophenol, a hydrolysis product of TCPO.[16] Therefore, bis(4-methoxycarbonyl-2-nitrophenyl) oxalate (**Ia**) was first synthesised. Next, to produce the affinity of the oxalates to the hydrophilic solvents, the ethylene glycol moiety was attached to the carboxy group of the parent compounds to give **Ib, Ic, IIb, IIc** and **IId** (Fig. 1).

## Synthesis

To 4-hydroxy-3-nitrobenzoic acid(**III**) (11.0 g, 60.1 mmol) in dry benzene (50 ml) containing dry pyridine (5 drops) was added dropwise thionyl chloride (9.20 g, 77.3 mmol) at 50–60 °C. (**Caution**—Benzene is highly toxic and appropriate precautions should be taken.) After stirring for 4 h, the reaction mixture was condensed under vacuum to give an oily residue of 4-hydroxy-3-nitrobenzoyl chloride (**IIIa**),[17] 12.0 g, yield 99.2%. IR (neat): 1750 $cm^{-1}$ (–COCl) and 3250 $cm^{-1}$ (Ar–OH).

The residue (**IIIa**, 12.0 g, 59.5 mmol) was dissolved in ethylene glycol monomethyl ether (22.8 g, 230 mmol) and heated at 70–75 °C for 2 h. The reaction mixture was evaporated under vacuum to give methoxyethyl 4-hydroxy-3-nitrobenzoate (**Vb**) as yellow crystals, 14.1 g, yield 98.2%, m.p. 49–51 °C. IR (KBr): 1725 $cm^{-1}$ (CO–O) and 3250 $cm^{-1}$ (Ar–OH). NMR ($CDCl_3$): δ 3.5 (3H, s, O–$CH_3$), 4.5 (2H, t, –$COOCH_2$–), 10.8 (1H, s, –OH), 3.8 (2H, t, –$CH_2O$–) and 7.2–8.9 p.p.m. (3H, m, $C_6H_3$). UV (acetonitrile): $\lambda_{max.}$ 240 nm ($\varepsilon = 3.90 \times 10^4$ l $mol^{-1}$ $cm^{-1}$).

**Vb** (4.80 g, 19.9 mmol) and triethylamine (2.20 g, 21.7 mmol) were dissolved in dry benzene (50 ml) and to this solution was added dropwise oxalyl chloride (1.13 g, 8.90 mmol) at 5–8 °C under a stream of nitrogen. After reaction for 3 h, the resulting precipitates were collected and dissolved in dry benzene. The filtrate was evaporated under vacuum to half the volume to yield pale yellow prisms, **Ib** (3.60 g, yield

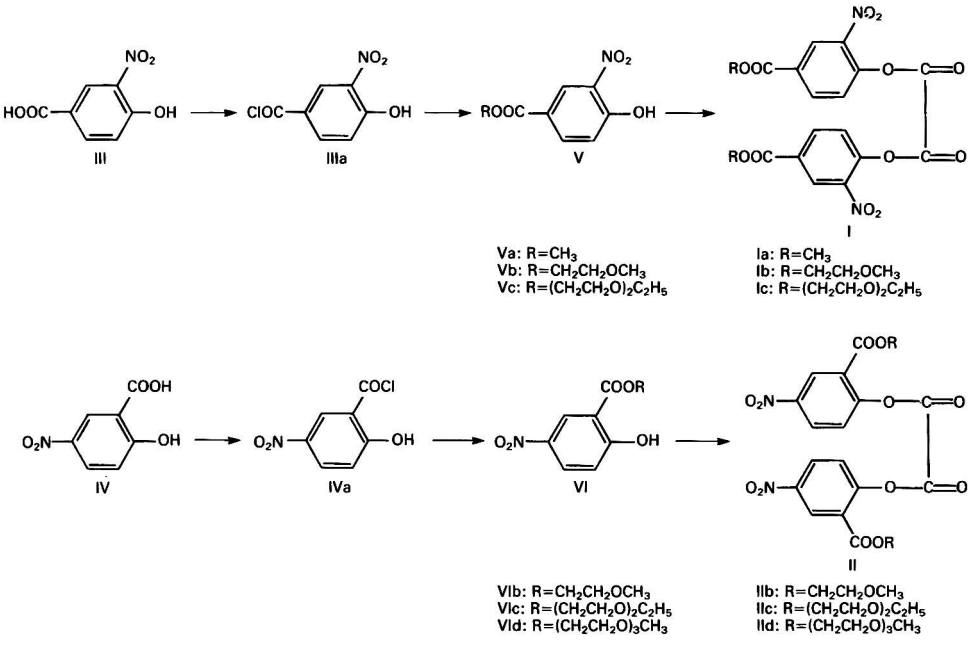



**Fig. 1.** Synthetic route for the new oxalates

**Table 1.** Physico-chemical properties of the new oxalates. $^1$N NMR spectra were recorded on a JEOL Model PMX-60-SI spectrometer at 60 MHz using tetramethylsilane as an internal standard. Abbreviations: s, singlet; t, triplet; and m, multiplet. IR spectra were recorded using KBr discs with a Jasco Model IRA-2 spectrometer. UV spectra were measured with a Hitachi-557 spectrophotometer

| Compound | Appearance | M.p./°C | Formula | Analysis, % C | H | N | Absorption spectra (in $CH_3CN$) $\lambda_{max.}$/nm | $\varepsilon \times 10^4$/l mol$^{-1}$ cm$^{-1}$ | IR (KBr)/cm$^{-1}$ | $^1$H NMR ($CDCl_3$), p.p.m. | Solubility (at 25 °C)/mM $CH_3CN$ | $CH_3COCH_3$ | $C_2H_5OAc$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Ia** | Colourless powder | 167–169 | $C_{18}H_{12}O_{12}N_2$ | | | | | | | | | | |
| | | | Calcd. | 48.23 | 2.70 | 6.25 | 236 | 4.26 | 1790 | 4.0, s, 6H | 16 | 18 | 6 |
| | | | Found | 47.69 | 2.64 | 6.03 | 340 | 0.54 | 1725 | 7.2–9.0, m, 6H | | | |
| | | | | | | | | | 1530 | | | | |
| | | | | | | | | | 1350 | | | | |
| **Ib** | Pale yellow prisms | 114–116 | $C_{22}H_{20}O_{14}N_2$ | | | | | | | | | | |
| | | | Calcd. | 49.26 | 3.76 | 5.22 | 233 | 3.90 | 1780 | 3.4, s, 6H | 58 | 71 | 35 |
| | | | Found | 49.34 | 3.61 | 5.19 | 340 | 0.41 | 1725 | 3.7, t, 4H | | | |
| | | | | | | | | | 1535 | 4.5, t, 4H | | | |
| | | | | | | | | | 1360 | 7.3–8.9, m, 6H | | | |
| **Ic** | Pale yellow crystals | 97–99 | $C_{28}H_{32}O_{16}N_2$ | | | | | | | | | | |
| | | | Calcd. | 51.54 | 4.94 | 4.29 | 232 | 3.88 | 1780 | 1.2, t, 6H | 273 | 192 | 83 |
| | | | Found | 51.61 | 5.32 | 4.26 | 340 | 0.39 | 1725 | 3.6, m, 16H | | | |
| | | | | | | | | | 1535 | 4.5, t, 4H | | | |
| | | | | | | | | | 1350 | 7.3–8.9, m, 6H | | | |
| **IIb** | Colourless needles | 126–128 | $C_{22}H_{20}O_{14}N_2$ | | | | | | | | | | |
| | | | Calcd. | 49.26 | 3.76 | 5.22 | 222 | 3.77 | 1770 | 3.4, s, 6H | 52 | 58 | 17 |
| | | | Found | 48.92 | 3.62 | 5.00 | 296 | 1.44 | 1720 | 3.7, t, 4H | | | |
| | | | | | | | | | 1530 | 4.5, t, 4H | | | |
| | | | | | | | | | 1360 | 7.4–9.0, m, 6H | | | |
| **IIc** | Almost colourless crystals | 89–90 | $C_{28}H_{32}O_{16}N_2$ | | | | | | | | | | |
| | | | Calcd. | 51.54 | 4.94 | 4.29 | 221 | 3.83 | 1770 | 1.2, t, 6H | 329 | 213 | 115 |
| | | | Found | 51.02 | 5.43 | 4.09 | 296 | 1.46 | 1720 | 3.6, m, 16H | | | |
| | | | | | | | | | 1530 | 4.5, t, 4H | | | |
| | | | | | | | | | 1350 | 7.3–9.0, m, 6H | | | |
| **IId** | Colourless crystals | 65–67 | $C_{30}H_{36}O_{18}N_2$ | | | | | | | | | | |
| | | | Calcd. | 50.56 | 5.09 | 3.93 | 220 | 3.90 | 1780 | 3.4, s, 6H | 1009 | 767 | 359 |
| | | | Found | 50.94 | 4.81 | 3.78 | | | 1710 | 3.7, m, 20H | | | |
| | | | | | | | | | 1530 | 4.5, t, 4H | | | |
| | | | | | | | | | 1350 | 7.5–9.0, m, 6H | | | |
| DNPO | Colourless powder | 192–194 | $C_{14}H_6O_{12}N_4$ | | | | | | | | 20 | 36 | 8 |
| TCPO | Colourless powder | 196–198 | $C_{14}H_4O_4Cl_6$ | | | | | | | | 3 | 14 | 13 |

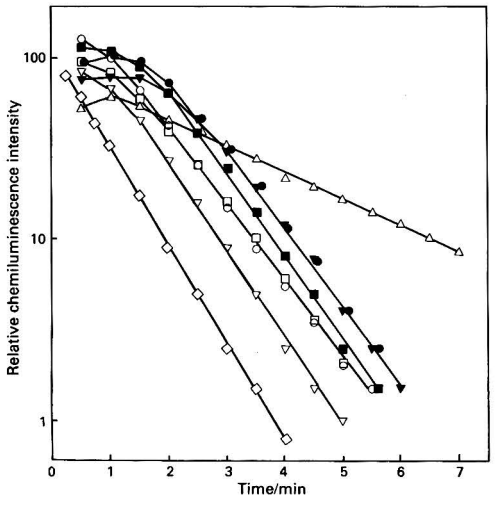



**Fig. 2.** Chemiluminescence reaction curves obtained from the new oxalates, DNPO and TCPO. A 1-ml volume of 10 μM perylene in acetone and 0.1 ml of 10 mM $H_2O_2$ in 50 mM imidazole - nitrate buffer (pH 6.50) were mixed in a quartz cuvette (1 × 1 × 5 cm). To this solution were added 1 ml of a 1 mM solution of each oxalate in acetonitrile. The generated chemiluminescence was measured with a fluorophotometer (UM-2S, Kotaki Seisakusho, Tokyo, Japan) with the light source off. Graphs: ○, **Ia**; ▽, **Ib**; □, **Ic**; ▼, **IIb**; ■, **IIc**; ●, **IId**; △, TCPO; and ◇, DNPO

67.1%, m.p. 114–116 °C). IR (KBr): 1780 $cm^{-1}$ (CO–CO). NMR ($CDCl_3$): δ 3.4 (6H, s, O–$CH_3$), 3.7 (4H, t, –$CH_2O$–), 4.5 (4H, t, –$COOCH_2$–) and 7.2–8.9 p.p.m. (6H, m, $C_6H_3$). UV (acetonitrile): $\lambda_{max.}$ 233 nm ($\varepsilon = 3.90 \times 10^4$ l $mol^{-1}$ $cm^{-1}$). Analysis: calculated for $C_{22}H_{20}O_{14}N_2$: C 49.26, H 3.76, N 5.22; found, C 49.34, H 3.61, N 5.19%.

The other oxalate esters **Ia, Ic, IIb, IIc** and **IId** were synthesised via the intermediates **Va, Vc, VIb, VIc** and **VId** in a similar manner (Fig. 1).

## Results and Discussion

The physico-chemical properties of the oxalate esters are given in Table 1. It is clear that the hydrophilicity was enhanced as the number of ethylene glycol moieties in the ester increased. The synthesis of an extremely soluble oxalate in the hydrophilic solvent was attained by the introduction of the triethylene glycol moiety to the skeleton of **IV** to give **IId**.

The introduction of the tetraethylene glycol moiety would be more preferable for enhancing the solubility further, but pure tetraethylene glycol was not commercially available.

The chemiluminescence reaction curves for **Ia, Ib, Ic, IIb, IIc** and **IId** and also DNPO and TCPO are shown in Fig. 2. Within 2 min all the new oxalates gave the maximum intensity and decreased their chemiluminescence intensities at a similar rate. Under the experimental conditions, the new oxalates gave higher chemiluminescence intensities than those given by DNPO and TCPO.

In preliminary experiments, the stabilities of **IIb, IIc** and **IId** in the presence of hydrogen peroxide[18] were superior to those of **Ia, Ib** and **Ic**, which means that the type of oxalates having the structure of **VI** might be well suited for use in the post-column reaction in HPLC.[8–16]

When the concentration of the oxalate **IId** in the medium was increased in proportion to that of hydrogen peroxide, the relative chemiluminescence intensities increased proportionally to the concentration (the relative maximum intensities are 1, 4.27, 10.8 and 16 for 0.5, 1.0, 2.0 and 3.0 mM **IId**, respectively), meaning that the enhanced solubility of the oxalates would be helpful for more sensitive detection in the peroxyoxalate reaction. The application of **IId** to the detection system for HPLC is currently being studied and the details will be published elsewhere.

In conclusion, a novel series of oxalate esters having hydrophilic properties have been developed, and especially bis[4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl] oxalate, **IId**, which is extremely soluble in acetone, acetonitrile and ethyl acetate, is expected to be useful for the high-sensitivity detection of both hydrogen peroxide and fluorescent compounds.

## References

1. Rauhut, M. M., Bollyky, L. J., Roberts, B. G., Loy, M., Whitman, R. H., Iannotta, A. V., Semsel, A. M., and Clarke, R. A., *J. Am. Chem. Soc.*, 1967, **89**, 6515.
2. Rauhut, M. M., *Acc. Chem. Res.*, 1962, **2**, 80.
3. Mohan, A. G., and Turro, N. J., *J. Chem. Educ.*, 1974, **51**, 528.
4. Williams, D. C., Huff, G. F., and Seitz, W. R., *Anal. Chem.*, 1976, **48**, 1003.
5. Williams, D. C., and Seitz, W. R., *Anal. Chem.*, 1986, **48**, 1478.
6. Arakawa, H., Maeda, M., and Tsuji, A., *Chem. Pharm. Bull.*, 1982, **30**, 3036.
7. Curtis, T. G., and Seitz, W. R., *J. Chromatogr.*, 1977, **134**, 343.
8. Kobayashi, S., and Imai, K., *Anal. Chem.*, 1980, **52**, 424.
9. Mellbin, G., *J. Liq. Chromatogr.*, 1983, **6**, 1603.
10. de Jong, G. J., Lammers, N., Spriit, F. J., Brinkman, U. A. Th., and Frei, R. W., *Chromatography*, 1984, **18**, 129.
11. Kobayashi, S., Sekino, J., Honda, K., and Imai, K., *Anal. Biochem.*, 1981, **112**, 99.
12. Mellbin, G., and Smith, B. E. F., *J. Chromatogr.*, 1984, **312**, 203.
13. Sigvardson, K. W., and Birks, J. W., *Anal. Chem.*, 1983, **55**, 432.
14. Weinberger, R., Mannan, C. A., Cerchio, M., and Grayeski, M. L., *J. Chromatogr.*, 1984, **288**, 445.
15. Sigvardson, K. W., Kennish, J. M., and Birks, J. W., *Anal. Chem.*, 1984, **56**, 1096.
16. Honda, K., Sekino, J., and Imai, K., *Anal. Chem.*, 1983, **53**, 940.
17. Wagner, G., and Singer, D., *Pharm. Zentralbl.*, 1964, **103**, 794; *Chem. Abstr.*, 1965, **63**, 9937d.
18. Honda, K., Miyaguchi, K., and Imai, K., *Anal. Chim. Acta*, in the press.

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# Accurate Determination of Platinum, Palladium, Gold and Silver in Ores and Concentrates by Wet Chemical Analysis of the Lead Assay Button

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A combined fire assay - wet chemical procedure for the accurate and rapid determination of platinum, palladium, gold and silver is described. The sample is fused with lead flux and the lead button is parted with perchloric acid after heating at 180–190 °C. After dilution of the parting solution with water, a formic acid reduction step is applied for the selective group precipitation of the four noble metals. After filtration, the precipitate is dissolved in aqua regia and the noble metals are determined by atomic absorption spectrometry. The proposed method was applied to a variety of sample types and, compared with the classical lead cupellation method, showed greater accuracy and precision in all instances.

**Keywords:** *Platinum, palladium, gold and silver determination; lead collection; perchloric acid parting; formic acid reduction - precipitation; atomic absorption spectrometry*

In earlier papers it was shown that following a controlled perchloric acid parting of the lead - noble metals button (180–190 °C) the platinum, palladium, rhodium and gold in the lead perchlorate parting solution could be simultaneously precipitated by 2-mercaptobenzothiazole[1] or by thiobarbituric acid,[2] and then removed by filtration. An agreeable feature of the perchloric acid parting is that complete precipitation of silver, as silver chloride, is attained by diluting with water and overnight standing of the lead perchlorate parting solution[3]; silver can thus be easily removed at an early stage in the analysis of the lead - noble metals button and so will not pose procedural difficulties in the subsequent determination of the platinum metals. The major advantage, however, of the perchloric acid parting technique is that the resulting parting solution containing the platinum, palladium, rhodium and gold, after being diluted with an equal volume of water, behaves as a non-oxidising medium, from which the noble metals can be removed by using suitable organic precipitants, including certain common reducing agents. This latter example has been demonstrated clearly in a recent paper from this laboratory,[4] which describes the complete precipitation of gold in the lead perchloric acid parting solution by reduction with formic acid or hydroquinone. In the course of the above investigation it was observed that when gold was determined in samples also containing platinum metals, the metallic gold precipitate was accompanied by high proportions of platinum and palladium and small amounts of rhodium. This coprecipitation encouraged further research with a view to extending the scope of the above-mentioned reduction technique to platinum and palladium. As platinum, palladium, gold and silver account for the major value of the precious metal content in almost all of the platiniferous materials, and as these four noble metals are usually determined on a routine basis, the development of a rapid and accurate non-cupellation method for their determination is highly desirable.

This paper describes the optimum conditions for the above purpose, with formic acid as a reductant.

## Experimental

### Equipment and Reagents

A fire assay electric furnace with a silicon carbide element, fireclay crucibles and cast-iron conical moulds (150-ml capacity) were used for the lead fusion. A Techtron Model AA-375 atomic absorption spectrometer was used for analytical measurements.

*Lead flux.* This contained: litharge, 50; disodium tetraborate (borax), 20; soda ash, 60; silica, 10–20 (depending on silica in the sample); and flour, 5–6 parts by mass.

*Formic acid*, 98–100%.

*Uranium buffer solution.* Prepared by dissolving 29.5 g of $U_3O_8$ in 20 ml of aqua regia and diluting to 100 ml with water.

### Recommended Procedure

Weigh 1–50 g of the sample and roast it over a thin silica bed in a shallow fireclay dish at 750 °C to convert the sulphides into oxides. Mix the roasted sample with 150–180 g of lead flux in a fireclay fusion crucible of suitable size. Fuse at 1200 °C for 1 h and then pour the molten fluid into a conical iron mould. After cooling, detach the lead button from the slag by tapping. Place the button in 100 ml of 30% *m/V* sodium hydroxide solution and boil to remove completely any adhering slag.

Place the clean lead button (30–60 g) in an 800-ml beaker and add 250 ml of 70% perchloric acid and 25 ml of glacial acetic acid. Cover the beaker, heat to 185 °C initially, and then maintain the parting temperature at 180–190 °C until all the lead has dissolved. Continue heating for another 20 min at a lower temperature of 150 °C. Allow to cool to approximately 100 °C and dilute the lead perchlorate solution with 250 ml of water while stirring (a white turbidity indicates the precipitation of silver chloride). Bring the diluted solution to the boil and add slowly 20 ml of formic acid. Stir and continue boiling gently for 1 h (a black precipitate of metallic platinum and palladium and a pink precipitate of gold appear after 2–3 min of boiling). Cool in water for 15–20 min (if silver is also required, stir the cooled solution and leave to stand for 2 h in the dark). Filter the solution through a Millipore filter apparatus using a Millipore filter membrane (0.45 μm) and wash 3–4 times with water. Place the filter disc with the retained precipitate in the original 800-ml beaker, add 40 ml of aqua regia and boil until all the noble metals and filter disc are completely dissolved. Evaporate to dryness in the presence of a little sodium chloride. Add 20 ml of concentrated hydrochloric acid and 5–6 drops of hydrogen peroxide (100 volume) and boil gently. To the cooled hydrochloric acid solution add uranium buffer solution (to give a final concentration of 4% *V/V*) and dilute to the appropriate volume with 3 M hydrochloric acid (concentrations should be 35–200 p.p.m. of Pt, 35–200 p.p.m. of Pd, 5–20 p.p.m. of Au and 1–5 p.p.m. of Ag). Measure the Pt, Pd, Au and Ag by atomic absorption spectrometry (AAS) at 265.9, 340.4, 242.8 and 328.1 nm, respectively, after the preparation of suitable standard noble metal solutions.

## Results and Discussion

### Choice of Formic Acid as the Precipitant

A 40-g lead button obtained by fusing 10 mg of platinum powder with 150 g of lead flux was parted with perchloric acid and the solution was diluted with water as described under Recommended Procedure. The addition of 20 ml of formic acid resulted in a black precipitate after 3 min of boiling. The solution was kept boiling gently for 2 h. After cooling, the coagulated precipitate was filtered off and the efficiency of precipitation was checked by examining the filtrate for platinum. No platinum was detected in the filtrate.

A similar procedure to that described above for platinum was carried out for palladium. The addition of formic acid resulted in a black precipitate being formed almost instantly. The final filtrate was analysed for any unprecipitated palladium. Palladium could not be detected in the filtrate, thus confirming complete precipitation by formic acid under the conditions applied.

With respect to silver, the treatment of the parting solution with formic acid had an advantageous effect on the speed and coagulation of the precipitate. Not even trace amounts of silver were found in the filitrate after 1 h of standing, compared with the overnight standing required for the complete precipitation of silver chloride[3] in the absence of reductant.

Reductants other than formic acid were also tried. Hydroquinone failed to precipitate platinum completely and about 10% of this metal was found in the filtrate. Oxalic acid developed a black suspension after more than 1 h of boiling. Ascorbic acid produced a slimy black precipitate unsuitable for filtration.

As a result of these initial tests, formic acid was chosen for further experiments.

### Effect of Perchloric Acid Concentration

No differences were observed in the completeness and speed of precipitation of platinum and palladium for lead perchlorate parting solutions diluted with different volumes of water (250, 300 and 400 ml). As in practice 250 ml of perchloric acid (70%) are used to disintegrate the lead button, it would be practical to dilute the parting solution with an equal volume of water (250 ml) before attempting the reduction of the noble metals. Under these conditions, gold is also quantitatively precipitated.[4]

### Effect of Boiling Time on Precipitation

Tests on the effect of boiling time on the precipitation of platinum and palladium showed that boiling for 30 min was required for their quantitative precipitation. Gold required boiling for 45 min for its complete precipitation with formic acid.[4] A boiling time of 60 min was therefore chosen.

### Effect of Formic Acid Concentration

It was found that 10 ml of this reductant were sufficient for the quantitative precipitation of, at least, 10 mg of each noble metal after a boiling time of 60 min.

### Foreign Elements

The fate and effect of elements likely to be present in some noble metal-containing samples were also considered. Ni, Cu, Fe, Cr, Co, Zn, Bi, Te and Se were not precipitated by formic acid in the parting medium. Rh partially precipitated, but its presence had no effect on the AAS measurement of the noble metals concerned if uranium was used as a buffer.[5,6] Ir and Ru remained totally undissolved by the parting acid, while a portion of osmium was volatilised. It was, however, essential to remove any sulphur present in the sample, failing which a white turbidity of lead sulphate was introduced in the parting solution. This precipitation was easily eliminated by roasting the sample over a thin silica bed at 750 °C for 1 h before mixing it with the lead flux.

### Accuracy and Precision

A Rustenburg converter matte of known composition[2] was analysed in triplicate for platinum, palladium and gold. A 5-g mass of the sample was leached with 100 ml of 10 M hydrochloric acid, in the presence of 20 g of ammonium chloride, in order to remove the nickel, copper, iron and sulphur. The sample solution was diluted with 100 ml of water and filtered. The residue on the filter, containing all the platinum-group metals and gold, was fused with lead flux in the usual way. The three lead buttons were taken through the recommended procedure. The accuracy and reproducibility of the method were further evaluated by analysing six lead buttons obtained after fusion of lead fluxes spiked with synthetic mixtures containing the four noble metals in different ratios. The accurate and precise results from all these tests (Table 1) confirm the high efficiency of formic acid for the precipitation of these four noble metals from lead - perchloric acid solutions.

### Analysis of Various Samples and a Comparison with the Classical Lead Cupellation Method

Several platiniferous materials were analysed in triplicate for platinum, palladium, gold and silver by the lead - wet chemical method proposed and the values were compared with those obtained by the standard lead cupellation technique. The results, shown in Table 2, demonstrate the higher efficiency of the lead wet chemical method, and provide a general indication of the extent of the cupellation losses.

## Conclusions

This work has shown that when the lead - perchloric acid parting solutions containing platinum, palladium, gold and silver are diluted with an equal volume of water and heated to boiling in the presence of formic acid, the noble metals are readily precipitated. The nature of the precipitate is a particular advantage because it can be brought into solution by aqua regia attack and the resulting solution can be directly analysed by AAS. The proposed lead - wet chemical method is more accurate and precise than the classical fire assay because of the elimination of the cupellation. It is a more satisfactory method than those recorded variations of the classical fire

**Table 1.** Accuracy and precision

| Sample | Mass of metal/mg | | | |
|---|---|---|---|---|
| | Pt | Pd | Au | Ag |
| Converter matte* (three 5-g portions . . . . | 4.80 | 2.69 | 0.240 | — |
| | 4.82 | 2.69 | 0.237 | — |
| | 4.79 | 2.71 | 0.235 | — |
| Spiked lead fluxes: | | | | |
| 1† . . . . . | 1.994 | 4.992 | 1.002 | 1.990 |
| 2† . . . . . | 2.009 | 4.994 | 0.990 | 1.998 |
| 3† . . . . . | 1.998 | 5.002 | 1.004 | 2.000 |
| 4‡ . . . . . | 0.998 | 0.998 | 5.010 | 4.968 |
| 5‡ . . . . . | 1.002 | 1.000 | 4.964 | 5.004 |
| 6‡ . . . . . | 0.998 | 0.996 | 4.982 | 4.972 |

* Containing 4.79 mg of Pt, 2.68 mg of Pd and 0.239 mg of Au.

† Spiked with 2.006 mg of Pt, 5.000 mg of Pd, 0.998 mg of Au and 2.004 mg of Ag.

‡ Spiked with 1.003 mg of Pt, 1.000 mg of Pd, 4.990 mg of Au and 5.010 mg of Ag.

**Table 2.** Comparison of the results obtained by lead wet chemical and lead cupellation methods. All measurements were carried out by AAS

| Sample | Method | Amount of metal, p.p.m.* Pt | Pd | Au | Ag |
|---|---|---|---|---|---|
| Platinum ore . . . . | Pb wet chemical analysis | 4.35 | 1.73 | 0.26 | 0.51 |
| | Pb cupellation | 3.88 | 1.67 | 0.23 | 0.42 |
| Concentrate . . . . | Pb wet chemical analysis | 64.1 | 36.2 | 3.71 | 9.6 |
| | Pb cupellation | 61.8 | 34.9 | 3.55 | 8.0 |
| Converter matte . . | Pb wet chemical analysis | 956 | 530 | 47.5 | |
| | Pb cupellation | 934 | 526 | 46.3 | |
| Pt catalyst . . . . | Pb wet chemical analysis | 7130 | | | |
| | Pb cupellation | 7042 | | | |
| Electrolyte precipitate | Pb wet chemical analysis | 234 | 490 | 426 | 2030 |
| | Pb cupellation | 210 | 412 | 420 | 2010 |

* Average of three determinations.

assay employing reduction of the original size of the lead button (from 40 to 1 g) by scorification[9] or incomplete cupellation,[10–12] followed by parting the small lead button with nitric acid. Scorification and partial cupellation are not permissible techniques for accurate noble metal determinations.[13] The proposed method is also superior to the nickel sulphide fire assay method, which is an unsatisfactory collection method for gold[14–16] and silver[17] and perhaps for platinum.[15,16]

A possible use of the lead wet chemical method should be mentioned. In fire assay laboratories using the classical lead cupellation procedure, correction factors are generally applied to compensate for losses during cupellation; normally, the large numbers of samples analysed are of very similar composition. The proposed lead wet chemical method could serve as an arbitration method to establish accurate correction factors for the determination of platinum, palladium, gold and silver.

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## References

1. Diamantatos, A., *Anal. Chim. Acta,* 1977, **92**, 171.
2. Diamantatos, A., *Anal. Chim. Acta,* 1978, **98**, 315.
3. Diamantatos, A., *Anal. Chim. Acta,* 1983, **148**, 293.
4. Diamantatos, A., *Anal. Chim. Acta,* 1984, **165**, 263.
5. Mallet, R. C., Pearton, D. C. G., Ring, E. J., and Steele, T. W., *Talanta,* 1972, **19**, 181.
6. Scarborough, J. M., *Anal. Chem.,* 1969, **41**, 250.
7. Diamantatos, A., *Anal. Chim. Acta,* 1977, **90**, 171.
8. Diamantatos, A., *Anal. Chim. Acta,* 1977, **91**, 281.
9. Moloughney, P. E., *Talanta,* 1980, **27**, 365.
10. Kolosova, L. P., Novatskaya, N. V., and Vinnitskaya, E. G., *Zavod. Lab.,* 1976, **42**, 508.
11. Kolosova, L. P., *Zavod. Lab.,* 1982, **48**, 8.
12. Rakovskii, E. E., Zdorova, E. P., Kuligin, V. I., Popova, N. N., Fishkova, N. L., and Shvedova, N. V., *Zavod. Lab.,* 1982, **48**, 11.
13. Beamish, F. E., and Van Loon, J. C., "Analysis of Noble Metals, Overview and Selected Methods," Academic Press, New York, 1977, p. 185.
14. Robert, R. V. D., Van Wyk, E., Palmer, R., and Steele, T. W., *Nat. Inst. Metall., Repub. S. Afr., Rep.,* No. 1371, 1971.
15. Robert, R. V. D., Van Wyk, E., Palmer, R., and Steele, T. W., *J. S. Afr. Chem. Inst.,* 1972, **25**, 179.
16. Kuznetzov, A. P., Kukushkin, Y. N., and Makarov, D. F., *Zh. Anal. Khim.,* 1974, **29**, 2155.
17. Dixon, K., Jones, E. A., Rasmussen, S., and Robert, R. V. D., *Nat. Inst. Metall., Repub. S. Afr., Rep.,* No. 1714, 1975.

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# Gravimetric Determination of Iron by Precipitation as the $[(C_4H_9)_4N]_3[Fe(SCN)_6]$ Ion Pair

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The Fe(III) - $SCN^-$ complex forms a water-insoluble ionic association compound with the tetrabutylammonium ion. This compound shows a definite stoicheiometric composition, $[(C_4H_9)_4N]_3[Fe(SCN)_6]$, under the optimum conditions. The compound may therefore be used for the gravimetric determination of iron. The gravimetric factor, 0.0495, is more favourable than others reported elsewhere, permitting the determination of at least 0.60 mg of iron with an error in the order of 0.6–0.7%.

**Keywords:** *Gravimetry; iron determination; thiocyanate; tetrabutylammonium; ion pair*

There are only a few procedures for the gravimetric determination of iron,[1] and they have not been frequently used because of their low sensitivities and the difficulty in handling the precipitates. These gravimetric procedures include those in which the final weighed compound is iron(III) oxide; after precipitation with a reagent such as ammonia, urea (from homogeneous solution), benzoate, formate, cupferrate or hexamethylenetetramine, compounds with no definite stoicheiometric composition are obtained. Accordingly, it is necessary to heat until $Fe_2O_3$ (gravimetric factor = 0.6994) is obtained. Other procedures, in which diverse inorganic and organic compounds are weighed as the iron phosphate, $FePO_4$ (gravimetric factor = 0.3703), or iron quinolin-8-olate, $Fe(C_9H_6ON)_3$ (gravimetric factor = 0.1144), can also be mentioned.

In this work, the formation of an ion pair between the Fe(III) - $SCN^-$ complex and the $Bu_4N^+$ cation was studied in order to determine the optimum experimental conditions. After obtaining and drying the precipitate under these conditions, it showed a definite stoicheiometric composition and it proved possible to propose an experimental procedure for the gravimetric determination of iron in a definite range with acceptable precision.

## Experimental

### Reagents

All solutions were prepared with de-ionised, doubly distilled water.

*Fe(III) solutions.* Prepared from $Fe(NO_3)_3.9H_2O$ and standardised gravimetrically by precipitation with urea (homogeneous solution) and weighing as $Fe_2O_3$.

*Tetrabutylammonium solution,* 0.10 M. Prepared from tetrabutylammonium chloride ($Bu_4N^+Cl^-$) (Fluka and Ega Chemie).

*Ammonium thiocyanate.* Both the solid reagent itself and appropriate solutions were used.

*Other solutions.* Dilute nitric and sulphuric acid, ammonia and sodium acetate solutions.

*Qualitative tests and interferences.* For the qualitative tests and the study of interferences, solutions of various metallic cations were prepared from different water-soluble inorganic salts (analytical-reagent grade chemicals). Special mention should be made of the solutions of Cu(II), Au(III) and Pt(IV), prepared from the solid metals with purities of 99.88, 99.99 and 99.9%, respectively.

### Apparatus

A Stanton Model TR-01 thermobalance was used.

### Qualitative Tests

On adding the tetrabutylammonium salt to the blood-red Fe(III) - $SCN^-$ solution, in the presence of an excess of $SCN^-$, a bulky red precipitate was obtained. This was quickly deposited at the bottom of the test-tube, even at room temperature (20–25 °C), and it was unnecessary to cool below 10 °C as with other similar compounds.[2,3] This effect is indicative of the low water solubility of the ion pair.

Filtration was carried out through a sintered-glass crucible (porosity 4) and the residue washed with water at room temperature. The acidity of the initial solution did not appear to affect the quantitative precipitation.

### Procedure

By adding excesses of ammonium thiocyanate and tetrabutylammonium chloride to the Fe(III) solution, an insoluble red ion pair was obtained. The precipitate was separated by filtration, washed with water and dried to a constant mass.

## Results and Discussion

### Influence of the Drying Temperature

An excess of the tetrabutylammonium salt (35–40 ml of the 0.10 M aqueous solution) was added to each of the solutions containing 20.12 mg of iron(III) and an excess of ammonium thiocyanate (2–2.5 g of the solid); the precipitates were filtered, washed and dried at temperatures between 20 and 100 °C.

The mass was approximately constant up to 60 °C (Fig. 1), whereas the compound was destroyed at higher temperatures.

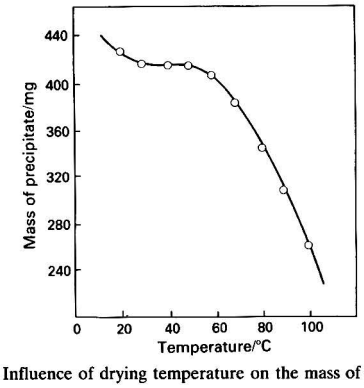


**Fig. 1.** Influence of drying temperature on the mass of precipitate formed

The decomposition of the quaternary ammonium salt above 60 °C was confirmed thermogravimetrically. After the precipitate had been dried at 40–50 °C to a constant mass, this mass did not increase when the solid was kept in the atmosphere at room temperature, *i.e.*, the precipitate did not show hygroscopic behaviour. In subsequent experiments the precipitate was therefore dried at 50 °C to a constant mass.

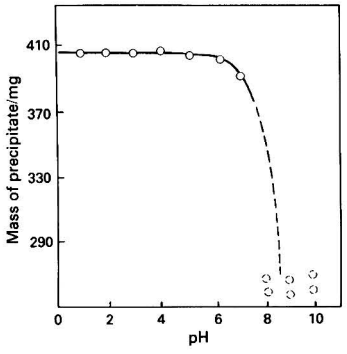


**Fig. 2.** Influence of pH on the precipitation. Fe(III) taken, 20.12 mg (0.360 mmol). ○, Irreproducible value

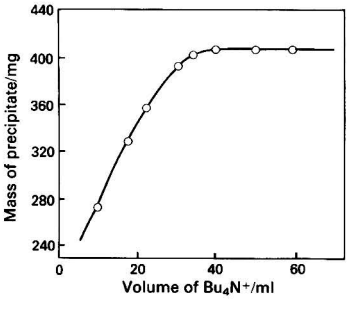


**Fig. 3.** Influence of volume of $10^{-1}$ M $Bu_4N^+$ solution on the precipitation. Fe(III) taken, 20.12 mg (0.360 mmol)

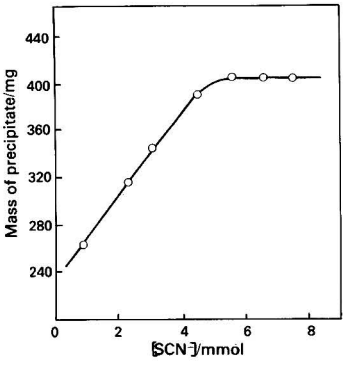


**Fig. 4.** Influence of $SCN^-$ concentration on the precipitation. Fe(III) taken, 20.12 mg (0.360 mmol)

### Influence of the $H^+$, $But_4N^+$ and $SCN^-$ Concentrations on Precipitation

Solutions containing the same amounts of iron(III) (20.12 mg) and $SCN^-$ (2–2.5 g of solid $NH_4SCN$) but with different pH values were prepared; after adding 35–40 ml of the 0.10 M $Bu_4N^+Cl^-$ solution, the respective ion pairs were obtained. The mass of the solids was nearly constant between pH 1 and 6 (Fig. 2), the thiocyanate complex being more stable than the insoluble hydrated oxide. At $pH < 1$, a redox reaction between Fe(III) and $SCN^-$ took place instead of formation of the complex. At $pH > 6$, the mass decreased because both the quaternary ammonium salt and the coordination complex became unstable in alkaline media.

These results agreed with those obtained for Fe(III) - $SCN^-$ solutions; at $pH \approx 5.5$ the intensity of the blood-red colour diminished and at a pH of nearly 8 the change to the insoluble hydrated oxide took place. After heating the precipitate obtained at $pH \geqslant 9$ to temperatures higher than 700 °C, the mass of the solid corresponded to that of the $Fe_2O_3$ obtained from 20.12 mg of iron.

To solutions containing 20.12 mg of iron(III) (0.360 mmol) and excess of ammonium thiocyanate (2–2.5 g), different amounts of the $Bu_4N^+Cl^-$ solution (varying from 1.0 to 6.0 mmol) were added. The mass was not constant until a value of the $Bu_4N^+$ to Fe(III) molar ratio between 10 and 12 was attained (Fig. 3). However, higher $Bu_4N^+$ concentrations were unsuitable because contamination of the precipitate increased and an undesirable change in the morphology of the solid took place, leading to a "soft" product.

Solutions containing 20.12 mg of iron(III) (0.360 mmol) and different amounts of $NH_4SCN$ ranging from 1.2 to 7.6 mmol were prepared at pH 2–3; on adding 35–40 ml of the 0.10 M $Bu_4N^+Cl^-$ solution, the corresponding insoluble ion pairs were obtained. The precipitates were separated, washed and dried at 50 °C. The mass was constant only from values of the $SCN^-$ to Fe(III) molar ratios of at least 15 (Fig. 4). The form and the colour of the precipitates were identical, probably because the compound separated was the same in all instances. However, a higher excesss of $SCN^-$ was preferable because it did not affect the morphology and because it contributed to a faster separation of the solid.

### Optimum Conditions: Determination of the Gravimetric Factor

The optimum conditions for precipitation were pH in the range 1.5–5, $NH_4SCN$ of the order of 2 g and between 35 and 40 ml of 0.10 M $Bu_4N^+Cl^-$ solution; these values were suitable for the amounts of Fe(III) studied in a total volume of nearly 100 ml.

Under these optimum conditions, the respective precipitates were obtained from solutions containing between 20.12 and 85.16 mg of iron(III) and the values of the experimental gravimetric factor were calculated. The values obtained were approximately the same (Table 1) and a mean value of 0.0495, corresponding to the stoicheiometric composition $[(C_4H_9)_4N]_3\,[Fe(SCN)_6]$, was calculated. This gravimetric factor is more favourable than all the others previously reported for the determination of iron.

### Range of Applicability, Precision and Accuracy of the Method

The gravimetric determination was carried out on samples with iron contents between 0.48 and 63.46 mg. Relative errors of the order of 0.6–0.7% for amounts higher than 0.60 mg and of the order of 0.3–0.5% for amounts higher than 10 mg of iron were obtained (Table 2).

By applying this method to 12 samples containing 20.12 mg of Fe, a mean value of $20.11_5$ mg was recorded and a relative standard deviation of 0.16% was calculated (Table 3).

**Table 1.** Determination of the experimental gravimetric factor

| Fe/mg | Precipitate/mg | Gravimetric factor |
|---|---|---|
| 20.12 | 406.5 | 0.0495 |
| 32.63 | 667.3 | 0.0489 |
| 41.90 | 839.7 | 0.0499 |
| 53.20 | 1079.1 | 0.0493 |
| 58.93 | 1207.6 | 0.0488 |
| 61.20 | 1226.5 | 0.0499 |
| 66.80 | 1333.3 | 0.0501 |
| 70.02 | 1420.3 | 0.0493 |
| 78.40 | 1574.3 | 0.0498 |
| 85.16 | 1720.4 | 0.0495 |
| | Mean: | 0.0495 |

**Table 2.** Range of applicability of the procedure

| Fe/mg | | |
|---|---|---|
| Taken | Found | Error, % |
| 0.48 | $0.48_5$ | +1.04 |
| 0.60 | 0.60 | +0.50 |
| 0.83 | 0.84 | +0.72 |
| 1.12 | 1.11 | −0.71 |
| 5.03 | 5.00 | −0.60 |
| 10.06 | 10.09 | +0.30 |
| 20.12 | 20.12 | — |
| 28.62 | 28.48 | −0.50 |
| 32.12 | 32.27 | +0.47 |
| 37.60 | 37.43 | −0.45 |
| 46.12 | 45.97 | −0.33 |
| 52.63 | 52.80 | +0.32 |
| 63.46 | 63.70 | +0.38 |

**Table 3.** Precision of the procedure. Fe taken: 20.12 mg

| Fe found/mg | $d$*/mg | Fe found/mg | $d$*/mg |
|---|---|---|---|
| 20.13 | $+0.01_5$ | 20.13 | $+0.01_5$ |
| 20.09 | $-0.02_5$ | 20.16 | $+0.04_5$ |
| 20.08 | $-0.03_5$ | 20.17 | $+0.05_5$ |
| 20.12 | $+0.00_5$ | 20.10 | $-0.01_5$ |
| 20.15 | $+0.03_5$ | 20.08 | $-0.03_5$ |
| 20.07 | $-0.04_5$ | 20.10 | $-0.01_5$ |

$\bar{x} = 20.11_5$ mg; $s = 0.0320$ mg; $s_r = 0.16\%$.
* $d$ = deviation = $x - \bar{x}$.

## Proposed Procedure

Dissolve a sample containing more than 0.60 mg of iron in the smallest amount of mineral acid possible (dilute $H_2SO_4$ or $HNO_3$ may be sufficient, although other mineral acids or mixtures of acids may be necessary in some instances) in a glass beaker. In order to ensure that complete oxidation of iron to iron(III) has been achieved, a test could be carried out by using the hexacyanoferrate(III) - iron(II) reaction. After removing the excess of acid, adjust the pH to between 1.5 and 5. Add between 2 and 2.5 g of $NH_4SCN$ and between 35 and 40 ml of 0.10 M $Bu_4N^+Cl^-$ solution at room temperature. Shake for several minutes, wait about 30–60 min and then separate the precipitate by filtration through a sintered-glass crucible (porosity 4). Wash the solid with several 10-ml portions of water at room temperature (20–25 °C) and dry at 50 °C to constant mass. The experimental gravimetric factor is 0.0495.

## Interferences

Other metal - thiocyanate complexes react with the tetrabutylammonium ion, causing turbidity; however, only in some instances is a true precipitate finally obtained, which settles slowly at the bottom of the beaker. Accordingly, Ce(III) gives rise to a slight greyish white opalescence and Mn(II), Zn(II), Cd(II), Hg(II) and Pb(II) produce white suspensions, although only in the last instance is a true precipitate obtained. Other water-insoluble ion pairs are obtained from Au(III) (rose white), U(VI) (light yellow), Pd(II) (orange - yellow), Pt(IV) (yellow), Bi(III) (orange - yellow), Ti(IV) (brownish red), V(IV) (light blue), Co(II) (blue) and Mo(V) and W(V) (red).

Some of these precipitates have an oil-like aspect and adhere easily to the walls of the container, affecting the quantitativeness of the precipitation; apparently only Fe(III), Ti(IV), Bi(III), Pd(II), Pb(II) and Cu(II) give rise to true precipitates, giving easily filterable granular solids. However, all the above mentioned elements interfere in the proposed gravimetric determination.

The minimum amounts of Pb(II), Cu(II), Bi(III), Pd(II), Ti(IV) and Au(III) that can be determined gravimetrically under the given experimental conditions are in the 3–5-mg range, with relative errors of the order of 1%.

As an example, from a sample containing 3.0 mg of Fe, 60.6 mg of the ion pair are obtained; the presence of the same amount (3.0 mg) of Pb(II), Cu(II), Bi(III), Pd(II), Ti(IV) or Au(III) causes positive errors, ranging approximately between 15% (for Pb or Au) and 60% (for Cu or Ti); these values should be treated with caution considering the small masses involved. The greater solubilities of the ion pairs of the other elements mentioned in the first paragraph allow them to be present at levels up to 20–30 mg without precipitation and without a subsequent interfering effect, except Ce(III), which can be present at levels up to 50 mg.

On increasing the amount of any one of the interfering elements, the error increases in proportion to the mass of the respective ion pair related to the Fe(III) ion pair and the content of iron itself. However, for smaller amounts of these metals it is very difficult to evaluate the errors because the precipitation is not quantitative.

It should be noted that Mo and W in their commonest oxidation state (+6) do not form precipitates that interfere in the process described.

Strong reducing agents, which convert Fe(III) to Fe(II), and oxidising agents capable of destroying the thiocyanate ion also interfere under the experimental conditions employed.

## References

1. Erdey, L., "Gravimetric Analysis, Part II," Pergamon Press, Oxford, 1965, p. 337.
2. Hernández-Méndez, J., Alonso-Mateos, A., Martín-Mateos, E. J., and García de María, C., *Rev. Roum. Chim.*, 1982, **27**, 981.
3. Hernández-Méndez, J., Alonso-Mateos, A., Martín-Mateos, E. J., and García de María, C., *Stud. Chem.*, 1984, **9**, 297.

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# SHORT PAPERS

## Size Distribution of Particulate Matter in Exhaust Gases in Inductively Coupled Plasma Atomic Emission Spectrometry

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An examination was made of the size of particulate matter in ICP-AES exhausts with the objective of defining the filtration requirements for glove-box installation. Using inactive simulants it was shown that >75% of the material is of sub-micrometre size with a median diameter in the range 0.1–0.2 μm. The presence of other elements does not alter significantly the size distribution of the major constituent.

**Keywords**: *Inductively coupled plasma; atomic emission spectrometry; exhaust gas analysis; particle size distribution*

During the last two decades, the inductively coupled plasma (ICP) has generally superseded d.c. arcs and r.f. sparks as sources for optical emission spectroscopy, in particular in the analysis of solutions, to the extent that ICP-AES is regarded as an established multi-element technique[1] for a wide range of materials. The proven reliability of the ICP makes its consideration for trace elements analysis in radioactive materials attractive, even though this introduces severe practical constraints, *e.g.*, in containment and cooling. Some consideration to the problem has been given,[2] although the experimental data on which to base a suitably designed and engineered facility is limited. This paper describes experiments using non-radioactive simulants on the particle size distribution in ICP-AES exhausts with a view to defining glove-box filtration requirements.

Owing to the complexity of the emission spectrum of either plutonium or uranium, it is necessary to separate the major constituent from the trace elements prior to analysis. However, no separation technique can be regarded as being capable of effecting complete separation, and the resulting portion containing the impurities must be regarded as containing residual activity. In the analysis of plutonium solutions, this residual activity is due to $^{241}Am$.

### Experimental

#### Apparatus

Details of the ICP system are given in Table 1, and are typical of the equipment and operating conditions for the torches and nebulisers of commercial ICP-AES systems. In this study the system is not sited in a glove-box.

A Casella five-stage cascade impactor was used to collect the particles from the exhaust; the first four stages are resin coated and the fifth is a cellulose acetate filter.

**Table 1.** ICP torch and nebuliser system

| | |
|---|---|
| Type | Plasmatherm Model HFP 2500D |
| Frequency | 27.12 MHz |
| Torch type | Demountable Fassel |
| Forward power/kW | 1.0–1.5 |
| Reflected power/W | 5 |
| Nebuliser | Perkin-Elmer fixed cross-flow |
| Coolant gas (Ar) flow-rate/l $min^{-1}$ | 15 |
| Auxiliary gas (Ar) flow-rate/l $min^{-1}$ | 0.3 |
| Nebuliser gas (Ar) flow-rate/l $min^{-1}$ | 0.6 at 26 p.s.i.a. |



All solutions were prepared from BDH Spectrosol standards using de-ionised water.

#### Procedure

The temperature of the exhaust gas at a height of *ca.* 175 mm vertically above the ICP plume was measured using a mercury thermometer. This represents the shortest distance at which it is possible to site a filter in the exhaust gas chimney.

The total gas flow through the chimney was determined from the analysis of its composition and a knowledge of the argon flow-rates through the torch. A warm-up period of 1 h was allowed before sampling of the gas using a stainless-steel pipe from the side and centre of the exhaust. Samples were analysed using a VG Micromass 8-80 mass spectrometer.

Dysprosium was used as an inactive simulant for $^{241}Am$, as the boiling-points of these two metals are similar, *viz.*, 2562 and 2607 °C for Dy and Am, respectively.[3] In addition, Dy ($\sigma_{Th}$, $^{164}Dy$ = 2100 b) can be determined rapidly by neutron-activation analysis with high sensitivity, and this was used to determine the amount collected at each stage of the impactor.

As analytical solutions contain impurity elements that could in principle alter the nucleation and growth characteristics of the major element present, the effect of additions of Cd (b.p. 756 °C), Mn (b.p. 1962 °C) and Ti (b.p. 3287 °C) at concentrations of 20 μg $ml^{-1}$ were also investigated. In order to simulate a plutonium - gallium alloy, one experiment was carried out with 1000 μg $ml^{-1}$ of gallium in solution.

Samples were collected using the impactor during the nebulisation of the appropriate solutions for times up to 2 min. The resin-coated filters from each stage were removed, rinsed with acetone and the washings collected in small polythene tubs. The final stage filter was placed in a similar tub and dissolved in acetone. All tubs were heated to dryness using an IR lamp, sealed and the Dy content was determined by instrumental neutron-activation analysis. For this the sealed tubs were irradiated in the Herald reactor for 1 min using the rapid transfer facility, and immediately after irradiation the induced $^{165}Dy^{m}$ activity ($t_{\frac{1}{2}}$ = 1.26 min) was measured by γ-spectrometry, the γ-photopeak at 108.2 keV being used for quantitative measurement. Under these conditions a limit of detection of 0.4 ng was achievable.

### Results and Discussion

#### Temperature

After operation for 1 h the maximum temperature observed was *ca.* 250 °C.

**Table 2.** Mass spectrometric analysis of exhaust gas

| Sampling point | Gas | Composition, % Run 1 | Run 2 |
|---|---|---|---|
| From side of ICP chimney . . | $N_2$ | 68.2 | 68.1 |
| | $O_2$ | 18.3 | 18.3 |
| | Ar | 13.2 | 13.3 |
| From centre of ICP chimney . . | $N_2$ | 68.1 | 68.1 |
| | $O_2$ | 18.4 | 18.5 |
| | Ar | 13.5 | 13.6 |

**Table 3.** Particle size distribution of material collected on the cascade impactor. Blank: < 0.4 ng

| Solution nebulised | Nebulisation time/min | Mass collected on stage/ng* 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| 10 μg ml$^{-1}$ Dy . . . . | 1 | 2 | 2 | 3 | 2 | 8 |
| 10 μg ml$^{-1}$ Dy . . . . | 2 | 3 | 3 | 4 | 4 | 21 |
| 100 μg ml$^{-1}$ Dy . . | 1 | 20 | 13 | 30 | 23 | 255 |
| 100 μg ml$^{-1}$ Dy + 20 μg ml$^{-1}$ Cd . . | 2 | 5 | 5 | 6 | 7 | 40 |
| 100 μg ml$^{-1}$ Dy + 20 μg ml$^{-1}$ Mn . . | 2 | 5 | 4 | 4 | 4 | 38 |
| 100 μg ml$^{-1}$ Dy + 20 μg ml$^{-1}$ Ti . . | 2 | 5 | 8 | 6 | 5 | 52 |
| 100 μg ml$^{-1}$ Dy + 1000 μg ml$^{-1}$ Ga | 2 | 2 | 3 | 4 | 5 | 76 |

* Stage 1 collects the coarsest material and stage 5 the finest.

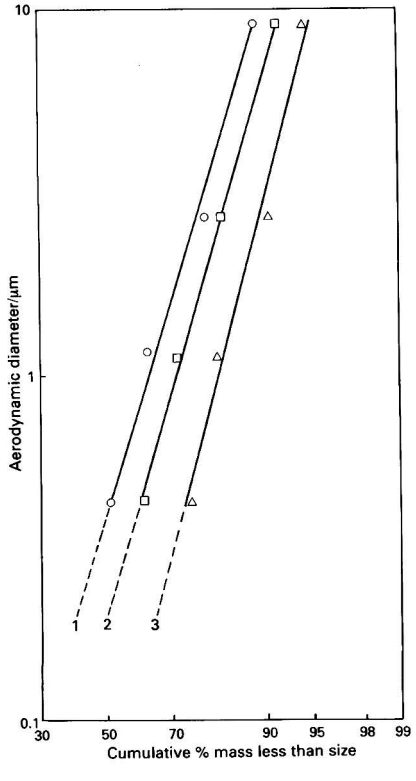


**Fig. 1.** Particle size distribution. Run: 1, 10 μg ml$^{-1}$ of Dy (1 min); 2, 10 μg ml$^{-1}$ of Dy (2 min); and 3, 100 μg ml$^{-1}$ of Dy (1 min)

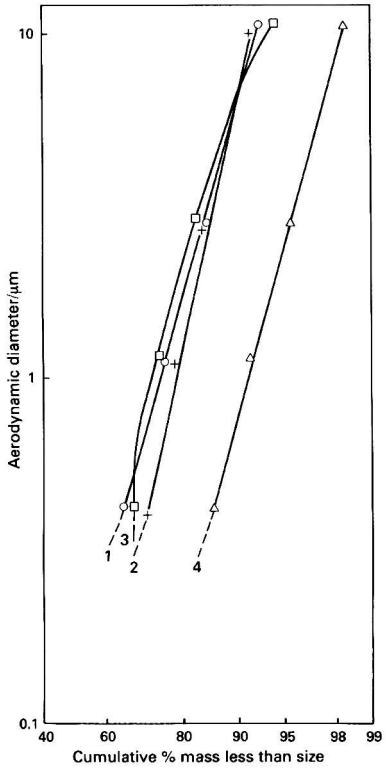


**Fig. 2.** Particle size distribution. Run: 1, 100 μg ml$^{-1}$ of Dy + 20 μg ml$^{-1}$ of Cd; 2, 100 μg ml$^{-1}$ of Dy + 20 μg ml$^{-1}$ of Mn; 3, 100 μg ml$^{-1}$ of Dy + 20 μg ml$^{-1}$ of Ti; and 4, 100 μg ml$^{-1}$ of Dy + 1000 μg ml$^{-1}$ of Ga

## Gas Dilution

Mass spectrometric data, given in Table 2, show good reproducibility and the absence of concentration gradients across the exhaust chimney (i.d. 70 mm). Taking these in conjunction with the gas flow-rates given in Table 1, and correcting for the Ar content of air, gives a total flow-rate of 130 l min$^{-1}$ through the exhaust chimney.

## Particulate Matter

The masses of Dy found at each stage of the impactor for each experiment are given in Table 3. Direct quantitative correlation between runs is not possible as the location of the sample probe above the plasma was not reproducible. Particle size distributions for the runs using solutions of Dy only and Dy solutions with known additions are given in Figs. 1 and 2, respectively. The main points emerging are as follows: (*i*) most of the particulate matter is collected on the final stage of the impactor; (*ii*) no significant differences are seen for different sampling times, thereby indicating that the sample is an equilibrium distribution; (*iii*) the particulate distribution changes slightly with concentration, the more concentrated solution producing the greater proportion of the finer material; (*iv*) the presence of elements at 20 μg ml$^{-1}$ concentration does not affect the Dy size distribution; (*v*) Ga, at 1000 μg ml$^{-1}$, alters the size distribution only slightly; and (*vi*) the mean diameter of the collected material is *ca.* 0.2 μm

for Dy solutions only; this reduces to *ca.* 0.1 μm in the presence of Ga.

## Conclusions

As an aid to the design of a filtration system for glove-boxes housing ICP-AES systems, this preliminary study has shown that a temperature of 250 °C exists at considerable distances from the plume, an air flow-rate of *ca.* 8 $m^3 h^{-1}$ is necessary for adequate cooling and particulate matter in the exhaust gas is mainly of sub-micrometre size with a median diameter in the range 0.1–0.2 μm. This median diameter occurs in the range in which the efficiency of HEPA filters is at a minimum.[4]

## References

1. Thompson, M., and Walsh, J. N., "Inductively Coupled Plasma Atomic Emission Spectroscopy," Blackie, Glasgow, 1983.
2. Apel, C. T., and Gallimore, D. L., *in* Lyon, W. S., *Editor*, "Proceedings of the 26th Conference on Analytical Chemistry in Energy Technology, Knoxville USA, October 11–13, 1983," Elsevier, Amsterdam, 1984, p. 213.
3. Weast, R. C., *Editor*, "CRC Handbook of Chemistry and Physics," Fifty-fifth Edition, CRC Press, Cleveland, OH, 1975.
4. Dyment, J., Process Technology Branch, AWRE, personal communication.

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# Sequential Determination of Arsenic, Antimony and Bismuth in Low-alloy Steels by Hydride Generation Inductively Coupled Plasma Atomic Emission Spectrometry

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The analysis of four NBS reference low-alloy steels for arsenic, antimony and bismuth is described. The method involves acid dissolution followed by hydride generation coupled with sequential determination by inductively coupled plasma atomic emission spectrometry. Chemical interferences were found to be negligible, and direct determination against synthetic standards was used. The method allows the rapid determination of the three elements in the same solution.

**Keywords:** *Arsenic, antimony and bismuth determination; hydride generation; inductively coupled plasma atomic emission spectrometry; low-alloy steels*

The introduction of a number of volatile hydrides into an inductively coupled plasma (ICP) and the simultaneous measurement of the optical emission has been described by Thompson.[1,2] This system uses a continuous flow apparatus to generate the hydrides, producing a steady-state signal. The technique should therefore be amenable to sequential determinations where a simultaneous ICP spectrometer is not available.

The individual analysis of six volatile hydride-forming elements in low-alloy steels by hydride generation atomic absorption spectrometry has been reported by Welz and Melcher.[3] In this method potential chemical interferences were minimised by dilution, as the method has adequate sensitivity to allow this.

A similar approach was adopted in the work reported here, using a hydride generator as described by Goulter[4] and a direct-reading sequential ICP spectrometer to determine all three analytes in the same solution.

## Experimental

### Apparatus

Measurements were carried out using an ARL 341 hydride generator connected to an ARL 3520 ICP spectrometer. Operating parameters of both are given in Table 1.

### Reagents

*Mixed stock solution of As, Sb and Bi.* Prepared from Spectrosol grade reagents (BDH Chemicals).

*Nitric acid, sp. gr.* 1.41. Aristar grade (BDH Chemicals).

*Hydrochloric acid, sp. gr.* 1.18. Aristar grade (BDH Chemicals).

*Sodium tetrahydroborate(III) solution,* 1% *m/V.* Prepared from Spectrosol grade reagent (BDH Chemicals) and stabilised with 0.1% *m/V* sodium hydroxide (analytical-reagent grade, BDH Chemicals) solution and filtered through a No. 3 sinter.

*Potassium iodide solution,* 50% *m/V.* Analytical-reagent grade.

### Procedure

Weigh accurately about 0.1 g of steel into a 100-ml beaker. Add 3 ml of hydrochloric acid and 1 ml of nitric acid. Heat gently to dissolve and dilute to about 50 ml. Add 15 ml of hydrochloric acid, cool and transfer into a 100-ml calibrated flask. Add 10 ml of potassium iodide solution and make up to volume.

Prepare standards in solutions of the same acid and potassium iodide concentration.

### Interferences

Chemical interferences were tested by checking recoveries of solutions containing 100 μg $l^{-1}$ of the three analytes plus levels of Cr, Cu, Mn, Mo, Ni and V at well above the maximum expected for low-alloy steels (Table 2).

As it has been reported[5,6] that iron inhibits the interference from elements such as nickel, owing to preferential reduction of Fe(III), the interference solutions also contained 1000 mg $l^{-1}$ of Fe, corresponding to the approximate level of iron in the steel solutions.

No significant suppressions were found and direct analysis could therefore be carried out. Mutual interferences between the hydride-forming elements have been shown not to occur in the ICP[2] and were not investigated.

**Table 1.** Operating parameters of the ICP spectrometer

| | |
|---|---|
| Incident power | 1200 W |
| Coolant gas flow-rate | 12 l $min^{-1}$ |
| Auxiliary gas flow-rate | 0.4 l $min^{-1}$ |
| Injector gas flow-rate | 1.0 l $min^{-1}$ |
| Observation height | 15 mm |
| Sample flow-rate | 6.1 ml $min^{-1}$ |
| Sodium tetrahydroborate flow-rate | 2.4 ml $min^{-1}$ |
| Wavelengths | As, 193.76 nm; Sb, 206.83 nm; Bi, 223.06 nm |

**Table 2.** Concentrations of interferents added to the sample solution

| Element | Concentration/ mg $l^{-1}$ |
|---|---|
| Cr | 30 |
| Cu | 7 |
| Mn | 150 |
| Mo | 50 |
| Ni | 200 |
| V | 20 |

**Table 3.** Certificated composition of other elements in the samples analysed. Results are in % *m/m*

| Sample | Cr | Co | Cu | Mn | Mo | Ni | W | V | Sn |
|---|---|---|---|---|---|---|---|---|---|
| NBS 361 | 0.694 | 0.032 | 0.042 | 0.66 | 0.19 | 2.00 | 0.017 | 0.011 | 0.010 |
| NBS 362 | 0.30 | 0.30 | 0.50 | 1.04 | 0.068 | 0.59 | 0.20 | 0.40 | 0.016 |
| NBS 363 | 1.31 | 0.48 | 0.10 | 1.50 | 0.028 | 0.30 | 0.046 | 0.31 | 0.104 |
| NBS 364 | 0.063 | 0.15 | 0.249 | 0.255 | 0.49 | 0.144 | 0.10 | 0.105 | 0.008 |

**Table 4.** Analysis of standard reference materials using the proposed technique. Results are in % *m/m*

| | As | | Sb | | Bi | |
|---|---|---|---|---|---|---|
| Sample | Certified value | Found | Certified value | Found | Certified value | Found |
| NBS 361 | 0.017 | 0.018 ±0.0001 | 0.0042 | 0.0043 ±0.0001 | (0.0004) | 0.0004 ±0.0001 |
| NBS 362 | 0.090 | 0.080 ±0.001 | 0.013 | 0.012 ±0.0005 | (0.002) | 0.0024 ±0.0002 |
| NBS 363 | 0.01 | 0.0094 ±0.0004 | 0.002 | 0.002 ±0.00008 | (0.0008) | 0.0005 ±0.0001 |
| NBS 364 | 0.052 | 0.040 ±0.001 | 0.034 | 0.033 ±0.0002 | (0.0009) | 0.0015 ±0.0002 |

## Results and Discussion

Four NBS standard steels were analysed (see Table 3 for major composition) and the results are given in Table 4. Where significant differences from the certificated values occur, *e.g.*, As in NBS 362 and 364, Bi in NBS 364, the results were similar to those obtained by Welz and Melcher.[3]

Limits of detection (2σ) for the method were 0.00003% of As, 0.00003% of Sb and 0.0001% of Bi, respectively, corresponding to 0.3 μg $l^{-1}$ of As, 0.3 μg $l^{-1}$ of Sb and 1 μg $l^{-1}$ of Bi in solution. Linear calibrations of up to 1 mg $l^{-1}$ were obtained for all three analytes.

The acid dissolution procedure gives a solution containing As(V) and Sb(V). Although it is possible to analyse these elements in the higher oxidation state against appropriate standards, the slower reduction leads to lower sensitivity in a continuous flow system, and reduction to the lower state with KI is preferred.

The hydride generation process involves the mixing of two highly reactive reagent streams and is thus inherently more noisy than conventional nebulisation. Relative standard deviations of 2–3% are typical at concentrations above the background equivalent. For this reason integration times of 10 s were employed. In order to minimise the analysis time, it is desirable to use a sequential spectrometer, which moves directly to the analytical wavelength, rather than performing a peak search operation. With duplicate integrations a sample may be determined in just over 2 min.

## Conclusion

The determination of As, Sb and Bi in low-alloy steels may be carried out directly by hydride generation ICP spectrometry. Direct analysis can be carried out and the use of a continuous flow system allows all three elements to be determined in the same solution.

## References

1. Thompson, M., Pahlavanpour, B., Walton, S. J., and Kirkbright, G. F., *Analyst*, 1978, **103**, 568.
2. Thompson, M., Pahlavanpour, B., Walton, S. J., and Kirkbright, G. F., *Analyst*, 1978, **103**, 705.
3. Welz, B., and Melcher, M., *Spectrochim. Acta, Part B*, 1981, **36**, 439.
4. Goulter, J. E., Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, 1981, Paper No. 129.
5. Fleming, H. D., and Ide, R. G., *Anal. Chim. Acta*, 1976, **83**, 67.
6. Welz, B., and Melcher, M., *Analyst*, 1984, **109**, 577.

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# Determination of Rock-forming Elements in the Presence of Large Amounts of Uranium and Zirconium Using Inductively Coupled Plasma Atomic Emission Spectrometry

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Inductively coupled plasma atomic emission spectrometry was used for the analyses of silicates containing major amounts of uranium and zirconium. A lithium metaborate fusion was used to dissolve the samples in which the following compounds were determined: silica, alumina, titania, iron oxide (total), lime, magnesia, uranium, manganese and zirconium.

Synthetic solutions were used for the calibration, and the instrumental stability was increased by the use of scandium as an internal standard.

**Keywords**: *Rock-forming element determination; uranium and zirconium interference; atomic emission spectrometry; inductively coupled plasma*

It is now accepted that inductively coupled plasma atomic emission spectrometry (ICP-AES) offers numerous advantages as a technique for the quantitative analysis of geological materials.[1-7] However, there are no available data on the determination of the common major elements in the presence of large amounts of Zr and U. These elements have extremely rich emission spectra and the probability of spectral overlap is therefore very high. As a result, a careful choice of line positions is necessary and particular care must be taken in the choice of the background positions used for corrections.

The method described uses a multi-channel inductively coupled plasma (ICP) spectrometer to determine all the elements usually required in a silicate analysis (except sodium and potassium) from one mother solution. Sodium and potassium may be determined in the same solution by atomic absorption spectrometry.

The amount of sample available was very limited and hence very small samples (20–50 mg) were dissolved using a lithium metaborate fusion.[8] This flux accommodated the large amounts of uranium and zirconium present. As an internal standard for ICP determination, 50 p.p.m. of Sc were added to samples and standards to improve short- and long-term precision.[2,3,9,10]

## Experimental

### Reagents

De-ionised water was used throughout

*Scandium internal standard solution.* Dissolve 1.53 g of scandium oxide in about 50 ml of nitric acid (5 N) and dilute to 1 l.

*Lithium metaborate.* Merck Spectromelt-A20, No. 12996.

*Calibration solutions.* Prepared for each element from analytical-reagent grade chemicals.

### Instrumentation

Instrument control and data manipulation were provided by a Digital Equipment Corporation PDP 11/03 computer with associated dual floppy disks.

*Spectrometer.* Jobin Yvon JY48 1-m vacuum multi-channel direct reading spectrometer with a holographic grating of 2550 lines $mm^{-1}$. Slit widths: entrance, 20 μm; exit, 50 μm. Resolution 0.012 nm.

*RF generator.* Plasmatherm, operating at 27 MHz with a forward power of 1.25 kW and less than 0.05 kW reflected.

*Plasma torch.* De-mountable assembly with Mermet configuration with three coaxial concentric tubes. The central injector is of alumina, the other tubes are made of silica glass.

*Nebuliser.* Meinhard glass concentric TR-C-20 high salt type, with humidifier on the argon aerosol carrier inlet.

*Gas flow-rates.* Coolant gas, 14 l $min^{-1}$; sheathing gas,* 0.4 l $min^{-1}$; and aerosol carrier gas, 0.7 l $min^{-1}$.

*Sample delivery.* Forced feeding, Minipuls II, peristaltic pump, 2 ml $min^{-1}$.

*Observation height.* Height, 12 mm above the work coil.

*Integration period.* Three periods of 10 s.

### Sample Preparation

The samples (50 mg) were fused with 250 mg of lithium metaborate ($LiBO_2$) in platinum - 5% gold crucibles over a gas flame. The cooled melt was dissolved in dilute nitric acid (1 N) and transferred into a 100-ml calibrated flask. Scandium internal standard solution was next added (5 ml) to make the amount of scandium in the final solution up to 50 μg $ml^{-1}$. The solution was then made up to volume.

In a number of instances, where insufficient sample was available, the amounts of reagents and volumes were varied. It was found that the final solution should contain 50 μg $ml^{-1}$ of scandium and that the ratio of sample mass to flux remained constant at 1:5.

### Standards and Calibration

The instrument was calibrated using multi-element synthetic standard solutions (Table 1). Separate standard solutions

**Table 1.** Composition of synthetic standard solutions, calculated as a percentage of the sample

| Compound | Amount in standard solution, % | | | | | |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI |
| $SiO_2$ | 10 | 80 | | | | |
| $Al_2O_3$ | 20 | 1 | | | | |
| $Fe_2O_3$ | 1 | 5 | | | | |
| CaO | 20 | 1 | | | | |
| MgO | 1 | 5 | | | | |
| $TiO_2$ | 0.45 | 4.5 | | | | |
| Mn | 0.1 | 10 | | | | |
| Sr | 0.01 | 1 | | | | |
| $U_3O_8$ | | | 1 | 80 | | |
| Zr | | | | | 1 | 10 |

* Sheathing gas is additional argon introduced between the spray chamber and torch to facilitate aerosol transport throughout the injector to the plasma exitation zone.[11]

**Table 2.** Analysis of standard uranium ore BL-5 by ICP spectrometry and a comparison with recommended values. All results are percentages

| | $SiO_2$ | $Al_2O_3$ | $TiO_2$ | Fe(total) | CaO | MgO | $U_3O_8$ | Mn | Zr | Sr |
|---|---|---|---|---|---|---|---|---|---|---|
| $n$ | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| $\bar{x}$ | 47.6 | 11.1 | 0.6 | 5.1 | 5.5 | 2.2 | 8.48 | 0.05 | 0.05 | 0.04 |
| R.s.d. | 0.4 | 0.2 | 0.3 | 0.8 | 1.0 | 0.4 | 0.9 | 0.4 | 4.5 | 0.6 |
| Recommended value | 47.1 | 11.3 | 0.7 | 5.8 | 5.6 | 2.3 | 8.36 | 0.05 | 0.04 | 0.03 |

**Table 3.** Composition of samples dissolved in lithium metaborate melts

| Component | Content, % |
|---|---|
| $SiO_2$ | 4–84 |
| $Al_2O_3$ | 0.5–7 |
| $TiO_2$ | 0.04–1.1 |
| $Fe_2O_3$ | 1–4 |
| CaO | 1–7 |
| MgO | 0.1–3 |
| $U_3O_8$ | 1–78 |
| Mn | 0.1–0.4 |
| Zr | 0.1–6 |

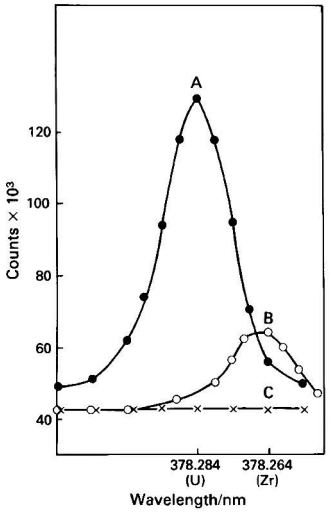


**Fig. 1.** Line interference of uranium by a secondary zirconium line. Graphs: A, U (100 p.p.m.); B, Zr (100 p.p.m.); and C, blank (water)

were used for zirconium and uranium because of the known mutual spectral interferences of these elements.[12,13] All calibration solutions contained the basic matrix elements derived from the dissolution procedure (*i.e.*, lithium metaborate and nitric acid). In addition solutions III–VI contained approximately 40% of $SiO_2$, 10% of $Al_2O_3$, 2% of $Fe_2O_3$ and 10% of CaO (calculated as a percentage of the solid sample).

## Choice of Wavelength

The wavelengths chosen for various elements were as follows: Si, 251.6; Al, 308.2; Fe, 259.9; Ca, 317.9; Mg, 279.5; Ti, 334.9; Mn 257.6; Sr, 407.7; U, 378.3; and Zr, 343.8 nm.

A systematic study was made of the structure of the analytical lines over a distance of ±0.05 nm from the peaks. Two principal interferences were found: 1 a secondary zirconium line on uranium; and 2, a secondary uranium line on the background position of aluminium.

### *Zirconium on uranium*

The wavelength chosen for the measurement of uranium was 0.003 nm from the optimum peak position in order to reduce the direct interference owing to the minor zirconium line at 378.264 nm (Fig. 1). Even with this offset it was found necessary to employ an empirical correction factor (applied by the instrument's computer) of 0.12% $U_2O_3$ for 1.0% of zirconium.

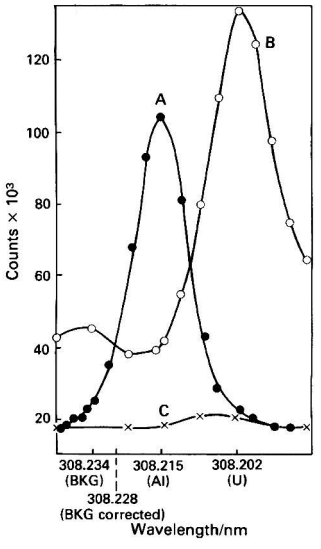


**Fig. 2.** Background interference of aluminium by a secondary uranium line. Graphs: A, Al (25 p.p.m.); B, U (400 p.p.m.); and C, blank (water)

### *Uranium on aluminium*

Particular care was necessary in the choice of the background correction position for the aluminium line. This was taken at +0.013 nm in order to minimise the effect of the secondary uranium peak on the background correction value. Background corrections on all other elements were made at +0.019 nm from the peak position (Fig. 2)

## Analysis

The samples were analysed using the ICP spectrometer in the usual way.[14]

## Results

The accuracy and precision of the method were tested by repeated analysis of a standard reference material, Canadian Standard BL-5. This material is a uranium ore and has been characterised by Faye *et al.*[15] Table 2 shows the results of six repeat analyses carried out on the same solution, and also the recommended values. As can be seen, the two sets of results are in excellent agreement. The stability of the instrument and the nebuliser used may be judged from the fact that the results in Table 2 were obtained over a period of 1.5 h.

## Discussion

The flux used in this method is capable of dissolving a wide range of uranium- and zirconium-containing rocks. Table 3 shows the range of materials dissolved in practice. About 20 rocks with widely differing compositions were used.

Disadvantages arising from the use of this flux are loss of lead owing to its volatility[16] and partial loss of some sulphur-containing species.[17]

On the whole the method represents a rapid and accurate way of analysing a difficult class of samples rich in uranium and zirconium.

## References


1. Church, S. E., *Geostand. Newsl.*, 1981, No. 2, 133.
2. Brenner, I. B., Watson, A. E., Russell, G. M., and Goncalves, M., *Chem. Geol.*, 1980, **28**, 321.
3. Watson, A. E., *ICP Inf. Newsl.*, 1979, No. 4, 441.
4. Burman, J. O., Ponter, C., and Bostrom, K., *Anal. Chem.*, 1978, **50**, 679.
5. Walsh, J. N., *Spectrochim. Acta, Part B*, 1979, **35**, 107.
6. Walsh, J. N., and Howie, B. A., *Mineral. Mag.*, 1980, **43**, 967.
7. Thompson, M., Ramsey, M. H., and Coles, B. J., *Analyst*, 1982, **107**, 1286.
8. Boar, P. L., and Ingram, L. K., *Analyst*, 1970, **95**, 124.
9. Russell, G. M., Mintek Rep. 2002, Randburg, 1979.
10. Schmidt, G. J., and Slavin, W., *Anal. Chem.*, 1982, **54**, 2491.
11. Trassy, C., Instruments S.A. Inc., *Fr. Pat.*, 79 15575, 1979.
12. Boumans, P. W. J. M., "Line Coincidence Tables for Inductively Coupled Plasma Atomic Emission Spectrometry," Pergamon Press, Oxford, 1980.
13. Parsons, M. L., Forster, A., and Anderson, D., "An Atlas of Spectral Interferences in ICP Spectroscopy," Plenum Press, New York, 1980.
14. Hoffer, D., Brenner, I. B., and Halicz, L., *ICP Inf. Newsl.*, 1984, No. 8, 494.
15. Faye, G. H., Bowman, W. S., and Sutarno, R., "Uranium Ore BL-5 a Certified Reference Material, Canment Report 79-4," Energy Mines and Resources Canada, Ottawa, 1979.
16. Johnson, M., and Maxwell, A., "Rock and Mineral Analysis," Wiley, Chichester, 1981, p. 302.
17. Johnson, M., and Maxwell, A., "Rock and Mineral Analysis," Wiley, Chichester, 1981, p. 160.


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# Comparison Between Isobutyl Methyl Ketone and Diisobutyl Ketone for the Solvent Extraction of Gold and Its Determination in Geological Materials Using Atomic Absorption Spectrometry

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A comparative study was made of the solvent extraction of the tetrachloro- and tetrabromoaurate complexes of gold into isobutyl methyl ketone and diisobutyl ketone. The efficiency of the extraction was assessed following the determination of residual gold in the aqueous phase by electrothermal atomic absorption spectrometry. Optimum conditions for solvent extraction were established. Sensitivity and precision data were gathered for gold extracted into isobutyl methyl ketone and diisobutyl ketone by flame atomic absorption spectrometry. Spectral interference data are presented for the co-extraction of iron.

**Keywords:** *Gold determination; solvent extraction; iron interference; atomic absorption spectrometry; geological materials*

The most commonly used method for the separation of gold from other elements in aqueous solution is based on the extraction of the tetrachloro- or tetrabromoaurate complex into 4-methylpentan-2-one (isobutyl methyl ketone, IBMK). The gold content is then measured by flame or electrothermal atomic absorption spectrometry (AAS).[1-4] It has been reported, however, that large concentrations of iron are co-extracted into IBMK and interfere as molecular absorption in the gold measurement.[1,2] To eliminate this interference it is necessary either to remove the iron by back-extraction or make background-corrected atomic absorption measurements.

Discussions with other participants at the Mineral Industry Research Organisation Gold Seminar in April 1984 indicated that 2,6-dimethylheptan-4-one (diisobutyl ketone, DIBK) has been gaining popularity as an alternative solvent to IBMK. A claim that cleaner separations are achieved and that co-extraction of iron is avoided prompted an investigation to assess whether the use of DIBK possesses any advantages over IBMK for the extraction of gold from geological matrix solutions.

## Experimental

### Apparatus and Operating Conditions

*Flame AAS measurements*

A Varian Techtron AA5 (updated to an AA6) atomic absorption spectrometer was used, under the operating conditions shown in Table 1.

*Electrothermal AAS measurements*

An Instrumentation Laboratory (IL) 151 atomic absorption spectrometer was used with an IL 555 electrothermal atomiser fitted with a single-piece pyrolytically coated graphite tube. Solutions were injected automatically into the graphite tube via an IL 254 Fastac autosampler. Real time absorbance peak heights were recorded on a Linseis chart recorder. The automated analytical programme is summarised in Table 2.

### Reagents and Solutions

All chemicals used were of analytical-reagent grade unless stated otherwise and distilled water was used throughout.

*Hydrochloric acid, sp. gr.* 1.18.

*Nitric acid, sp. gr.* 1.42.

*Hydrobromic acid, sp. gr.* 1.46–1.49.

*IBMK.* Equilibrated by shaking with 3 M HCl for 2 min.

*DIBK.* Technical grade. No gold was detected using graphite furnace AAS.

*Iron(III) chloride hexahydrate solution,* 5% *m/V.* Prepared in 3 M HCl for iron interference studies.

*Gold standard solutions,* 0.01, 0.02, 0.03 *and* 0.04 μg ml$^{-1}$. Prepared in 1% *V/V* HCl for electrothermal AAS calibration.

*Gold stock solution,* 100 μg ml$^{-1}$. A 100-mg amount of gold sponge (Johnson-Matthey Specpure grade) was dissolved in a mixture of 10 ml of hydrochloric acid and 2.5 ml of nitric acid and diluted to 1000 ml.

*Preparation of solvent extracts*

*Tetrachloroaurate.* A 50-ml volume of hydrochloric acid, containing 50 μg of gold, and 10 ml of organic solvent were shaken together for 1 min in a 100-ml separating funnel.

*Tetrabromoaurate.* A 50-ml volume of hydrochloric acid, containing 50 μg of gold, plus 2 ml of hydrobromic acid were mixed thoroughly and then shaken with 10 ml of organic solvent for 1 min in a 100-ml separating funnel.

After allowing the phases to separate the organic extracts were run off and stored in stoppered glass tubes.

## Results and Discussion

### Study of DIBK Volume Change

IBMK must be equilibrated before use. The change in volume of IBMK during equilibration is dependent on the acid concentration of the aqueous phase and the initial volumes of

**Table 1.** Operating conditions for flame AAS measurements

| | |
|---|---|
| Wavelength | 242.8 nm |
| Lamp current | 4 mA |
| Slit width | 100 μm |
| Air flow-rate | 6 l min$^{-1}$ |
| Acetylene flow-rate | 0.8 l min$^{-1}$ (IBMK); 1.2 l min$^{-1}$ (DIBK) |
| Solvent uptake rate | 4.8 ml min$^{-1}$ |
| Optimum burner heights: | |
| Tetrachloroaurate | 20 (IBMK); 13 (DIBK) |
| Tetrabromoaurate | 16 (IBMK); 10 (DIBK) |

**Table 2.** Summary of the graphite furnace atomisation programme in steps 1–6

| | Drying | | Ashing | | Atomisation | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Time/s | 0 | Deposition time | 20 | 20 | 0 | 5 |
| Temperature/°C | 0 | 175 | 450 | 600 | 2200 | 2200 |

the two phases.[5] A solubility study was made for DIBK for acidities between 1 and 6 M HCl and an aqueous to organic ratio of 5:1. All reagents were measured out at room temperature using standard calibrated glassware. After shaking there was a rapid, clean separation of the phases with no emulsion at the interface. Measurement of the phases after separation showed no change in volume.

### Efficiency of Extraction of Gold into IBMK and DIBK

The distribution coefficient for the extraction of gold from 3 M HCl into IBMK is quoted as greater than 1000.[6] The optimum acidity for the extraction of tetrabromoaurate into ketones is 2–3 M.[7] A 50-μg mass of gold was extracted into IBMK as the tetrachloroaurate and tetrabromoaurate complexes from solutions 3 M in HCl with greater than 99% efficiency in a single shaking. The percentage extraction of gold was assessed by determining the residual gold in the acid phase by graphite furnace AAS after dilution to give an acid strength between 1 and 5% *V/V* HCl. Within this range of acid concentration it had been found that there was no significant change in the absorbance of a standard gold solution.

The exercise was repeated and extended for DIBK. A 50-μg mass of gold in solutions ranging from 1 to 6 M in HCl was extracted into DIBK as the tetrachloroaurate and tetrabromoaurate complexes. The results are shown in Table 3.

The results indicate that the tetrabromoaurate extraction into DIBK is more complete than the tetrachloroaurate extraction, thus supporting the claim of Korkisch.[7] No improvement in the extraction efficiency of tetrachloro- or tetrabromoaurate at the 3 M HCl level was noted when (*a*) the shaking time was varied between 0.5 and 2 min; and (*b*) equilibrated DIBK was used for the extraction. An HCl concentration of 3 M was selected as the optimum acid concentration for solvent extraction and 1 min was chosen as the optimum shaking time.

**Table 3.** Efficiency of the extraction of 50 μg of gold into DIBK

| HCl concentration/M | Tetrachloroaurate extraction, % | Tetrabromoaurate extraction, % |
|---|---|---|
| 1 | 85.4 | 85.9 |
| 2 | 83.0 | 93.8 |
| 3 | 87.4 | 96.8 |
| 4 | 81.8 | 95.2 |
| 5 | 82.8 | 95.8 |
| 6 | 84.8 | 96.4 |

### Comparison of the Sensitivity and Precision of the Measurement of IBMK and DIBK Extracts

Table 4 compares the sensitivity and precision of the flame AAS determination of 50 μg of gold extracted from 3 M HCl into IBMK and DIBK as the tetrochloroaurate and tetrabromoaurate complexes. The extractions were all made on the same day and measured the following day (the extracts are stable for 48 h).

Better sensitivity and precision were obtained for gold extracted into IBMK. For both solvents the tetrabromoaurate measurements were more precise than those for the tetrachloroaurate complex.

### Co-extraction of Iron into IBMK and DIBK

A series of iron solutions were prepared in 3 M HCl to contain amounts of iron corresponding to a 5-g sample containing 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 g of Fe, *i.e.*, 5, 10, 15, 20, 25 and 30% of Fe (7–43% of $Fe_2O_3$).

These solutions were shaken with IBMK and DIBK and the resulting absorbances of the extracts are expressed in Table 5 as a percentage of the absorbance of a 50-μg gold extract.

Although the relative interference effect of iron is less when using DIBK it is not completely eliminated and can represent a significant contribution to the gold value.

The results obtained on measuring the tetrabromoaurate complex at the less sensitive wavelength of 267.6 nm are shown for comparison. Although reduced, some interference from co-extracted iron is still evident.

It was confirmed that the iron interference could be eliminated by hydrogen lamp background correction[1] or back-extraction with 0.1 M HCl (tetrachloroaurate)[1] or 0.1 M HBr (tetrabromoaurate).[2]

## Conclusion

On the strength of the above data there appears to be little advantage in using DIBK as opposed to IBMK for the extraction of gold from geological matrix solutions. The efficiency of extraction is generally less, the sensitivity and precision are poorer and there is still a significant effect of co-extracted iron especially at higher iron concentrations.

**Table 4.** Flame AAS determination of replicate extractions ($n$ = 6) of 50 μg of gold from 3 M HCl into IBMK and DIBK as the tetrachloro- and tetrabromoaurate complexes

| | IBMK | | DIBK | |
|---|---|---|---|---|
| | Tetrachloroaurate | Tetrabromoaurate | Tetrachloroaurate | Tetrabromoaurate |
| Absorbance range .. | 0.340–0.355 | 0.368–0.376 | 0.185–0.213 | 0.222–0.232 |
| Mean .. .. .. | 0.348 | 0.372 | 0.195 | 0.226 |
| RSD, % .. .. .. | 1.6 | 0.9 | 4.9 | 1.7 |

**Table 5.** Spectral interference of iron extracted from 3 M HCl expressed as a percentage of the absorbance of a 50-μg gold extract. Relative absorbance of 50 μg of Au = 100%

| | Relative absorbance, % | | | | | |
|---|---|---|---|---|---|---|
| | IBMK | | | DIBK | | |
| Mass of iron in a 5-g sample/g | Tetrachloroaurate, 242.8 nm | Tetrabromoaurate | | Tetrachloroaurate, 242.8 nm | Tetrabromoaurate | |
| | | 242.8 nm | 267.6 nm | | 242.8 nm | 267.6 nm |
| 0.25 | 16 | 16 | 7 | 1 | 1 | 0 |
| 0.50 | 23 | 26 | 10 | 1.5 | 3 | 1 |
| 0.75 | 30 | 32 | 12 | 3 | 6 | 2 |
| 1.00 | 35 | 36 | 13 | 5 | 10 | 4 |
| 1.25 | 38 | 41 | 15 | 9 | 15 | 7 |
| 1.50 | 40 | 44 | 16 | 12 | 20 | 8 |

Extraction of gold into IBMK, followed by back-extraction into dilute acid or background-corrected atomic absorption measurements, would seem to be the more reliable technique.

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## References

1. Strong, B., and Murray-Smith, R., *Talanta*, 1974, **21**, 1253.
2. Meier, A. L., *J. Geochem. Explor.*, 1980, **13**, 77.
3. Brooks, R. R., Holzbecher, J., Ryan, D. E., and Zhang, D. E., *At. Spectrosc.*, 1981, **2**, 151.
4. McHugh, J. B., *J. Geochem. Explor.*, 1984, **20**, 303.
5. Law, S. L., and Green, T. E., *Anal. Chem.*, 1969, **41**, 1008.
6. Strelow, F. W. E., Feast, E. C., Mathews, P. M., Bothma, C. J. C., and Van Zyl, C. R., *Anal. Chem.*, 1966, **38**, 115.
7. Korkisch, J., "Modern Methods for the Separation of Rarer Metal Ions," Pergamon Press, Oxford, 1969, p. 395.

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# Study of the Fluorescence of the Lead - Morin System in the Presence of Non-ionic Surfactants

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A study of the fluorescence of the Pb - morin system in the presence of the non-ionic surfactant Genapol PF-20 (ethylene oxide - propylene oxide condensate) has been made. The weak fluorescence of the Pb - morin system is enhanced about nine-fold, at a pH of 3.3 ± 0.2 with a surfactant concentration of 2%. The presence of a non-ionic surfactant also gives greater stability, the system being stable for at least 3 h. The excitation maximum occurs at 420 nm with fluoresence occurring at 495 nm.

The fluorescence is linear up to 1 μg ml$^{-1}$ of Pb, and the detection limit is found to be 0.06 μg ml$^{-1}$. The precision at 1 p.p.m. of Pb(II) is ±0.47%. Al(III), Be(II), Zn(II) and Sn(II) strongly increase the fluorescence, probably owing to the formation of fluorescent complexes with morin, which are highly sensitised by the surfactant used, and $F^-$, Fe(III) and Cu(II) decrease the fluorescence signal.

**Keywords:** *Lead - morin system; non-ionic surfactants; fluorescence*

There have been only a few reports concerning the application of micellar systems to fluorescence analytical determinations. The first application in this area concerned the improvement of a fluorimetric method for the determination of aluminium with lumogallion in the presence of the non-ionic surfactant polyethylene glycol monolauryl ether.[1] Recently, Sanz Medel and co-workers[2,3] and Hinze *et al.*[4] have revised the fluorimetric analytical applications in micellar media.

Using morin as a fluorimetric reagent, only the Al - morin,[5] Nb - morin[2,6] and Ta - morin[3] complexes have been sensitised by the use of surfactants. The fluorescence of the Al - morin complex has been strongly enhanced in the presence of the non-ionic surfactant Genapol PF-20 (ethylene oxide - propylene oxide condensate): the addition of 3% of Genapol enhanced the fluorescence intensity about 8–10 fold, producing an extremely sensitive method (detection limit, 0.2 p.p.b. of Al) for the fluorimetric determination of Al.[5]

From the interference of Pb(II) on the cited system (which does not interfere in the absence of surfactant) we deduced that the weak fluorescence of the Pb - morin system was significantly enhanced by the addition of Genapol PF-20. The aim of this work was to determine the optimum conditions in which the above-cited sensitisation occurs, and to determine the analytical characteristics of the system.

In contrast, the formation of the fluorescent complex between Pb and morin has not been extensively studied although in 1941 Goto proposed a test for Pb with morin in which as little as 0.25 μg of Pb was sufficient to obtain a yellow - green fluorescence.[7] Several years later, Pb(II) was identified and semi-quantitatively determined by the fluorescent reaction with morin after concentration by the ring-oven method.[8] Recently, two spectrophotometric studies were carried out[9,10] and the formation of Pb complexes with quercetin and morin was studied by spectrophotometry in aqueous ethanolic solutions (46%): two complexes were formed, $PbL^+$ and $PbL_2$, at pH 4.5 for morin and 6.0 for quercetin.[10]

## Experimental

### Apparatus

The fluorescence intensity measurements were made on a Perkin-Elmer 3000 spectrofluorimeter. Temperature checks were carried out using a Selecta Termotronic S-389 thermostat, which measures the temperature to ±0.5 °C. The pH was measured to a pH of ±0.001 with a Crison Digilab 517 pH meter.

### Reagents

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

*Lead stock solution* 1000 p.p.m. Prepared by dissolving 1.5984 g of $Pb(NO_3)_2$ (Merck) in 1 l of water. Working solutions were prepared by appropriate dilution with water.

*Surfactant.* Genapol PF-20 (Hoechst) with a propylene oxide - ethylene oxide ratio of 1:6.

*Working buffer solution, pH* 3.3. Prepared by mixing 13.1350 g of chloroacetic acid (Merck) with 5.2178 g of potassium hydroxide (Eka) and diluting with water to 1 l.

*Morin solution.* Prepared by dissolving the appropriate amount of reagent (Merck) in 100 ml of ethanol solution containing 9% of methanol and 5% of water.

## Results and Discussion

At first the influence of pH on the fluorescence intensity ($I_F$) of the lead - morin system in the absence of surfactant was studied. To obtain the optimum wavelengths, the excitation and emission spectra at different pH values were recorded. Fig. 1 shows the variation of $I_F$ with the pH (adjusted with $HNO_3$ - NaOH solution) for lead - morin and morin systems

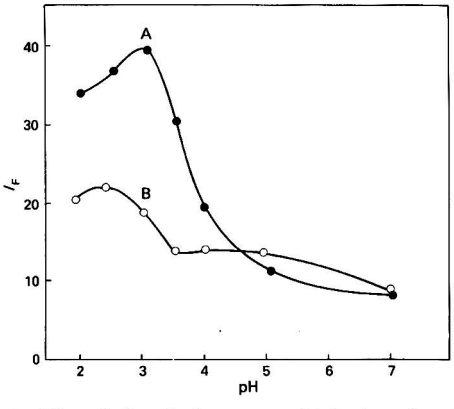


**Fig. 1.** Effect of pH on the fluorescence of A, lead - morin system; and B, morin. Conditions: lead, 2 μg ml$^{-1}$; morin, 0.01%

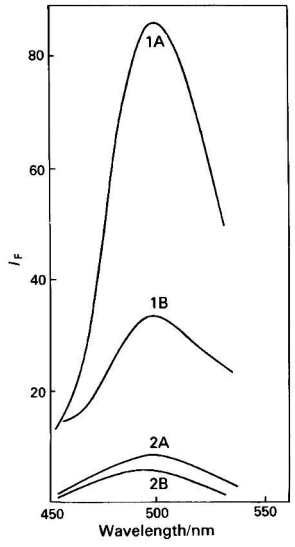


**Fig. 2.** Emission spectra of 1A, lead - morin - Genapol; 2A, lead - morin; 1B, morin - Genapol; and 2B, morin. Conditions: lead, 1 μg ml$^{-1}$; morin, 0.01%; Genapol PF-20, 2%; pH, 3.3 (chloroacetic acid - potassium hydroxide buffer; $\lambda_{ex}$, 420 nm

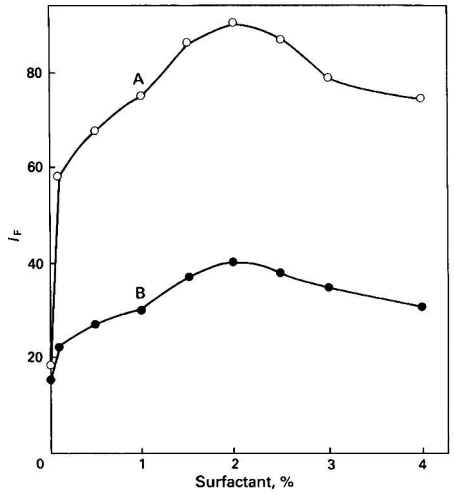


**Fig. 3.** Influence of surfactant concentration on the fluorescence of the lead - morin system. A, Lead - morin - Genapol PF-20; and B, morin - Genapol PF-20. Conditions: lead, 1 μg ml$^{-1}$; morin, 0.02%; pH 3.3 (chloroacetic acid - potassium hydroxide buffer); $\lambda_{ex}$, 420 nm; $\lambda_{em}$, 495 nm; time 1 h

over the pH range 2–7. It can be seen that the influence of pH is very severe, the fluorescence maximum being observed at a pH between 2 and 3. A maximum difference with the blank was obtained in the pH range 3.0–3.5. On the other hand, the fluorescence of the lead - morin system in the absence of surfactant was unstable and decreased with time.

It was found that the addition of Genapol PF-20 caused about a nine-fold enhancement and stabilisation of fluorescence. Fig. 2 shows emission spectra of the lead - morin system in the presence or absence of the surfactant, and those of the respective blanks (morin - Genapol and morin). The addition of the surfactant causes an important hyperchromic but not a bathochromic shift in the excitation and emission maxima. In both instances, the maximum fluorescence was obtained at 495 nm, corresponding to an excitation maximum of 420 nm.

**Table 1.** Effect of foreign ions on the determination of 0.2 μg ml$^{-1}$ of lead

| Ion | Limiting concentration/ ng ml$^{-1}$ | Ion | Limiting concentration/ ng ml$^{-1}$ |
|---|---|---|---|
| $CH_3COO^-$ | 800 | Ca(II) | >10000 |
| $Cl^-$ | >10000 | Co(II) | 5000 |
| $F^-$ | 80 | Cr(III) | >10000 |
| $NO_3^-$ | >10000 | Cu(II) | 25 |
| $PO_4^{3-}$ | 1000 | Fe(III) | 50 |
| $SiO_3^{2-}$ | 20 | Mg(II) | >10000 |
| $SO_4^{2-}$ | >10000 | Mn(II) | >10000 |
| Al(III) | 1 | $NH_4^+$ | >10000 |
| Ba(II) | >10000 | Ni(II) | 5000 |
| Be(II) | 5 | Sn(II) | 5 |
| | | Zn(II) | 10 |

## Effect of the Reaction Variables

The effect of the reaction variables on the fluorescence of the lead - morin - Genapol system was studied. The optimum pH was 3.3, adjusted with chloroacetic acid - potassium hydroxide buffer solution, and variations of ±0.25 were admissible without variation of the fluorescence of the system.

The influence of the concentration of Genapol PF-20 on $I_F$ was studied with fixed concentrations of morin (0.02%) and lead (1 μg ml$^{-1}$), with variable concentrations of surfactants between 0 and 4% *m/V*. It was observed that when the surfactant concentration increased, $I_F$ also increased until it reached 2%; from this value onwards a decrease in intensity was observed (Fig. 3). Moreover, when the surfactant concentration was less than 1%, the system was unstable, the fluorescence decreasing considerably with time; at concentrations higher than 1%, a major stabilisation was observed.

The effect of morin concentration (0.005–0.05%) at a fixed concentration of surfactant (2%) and lead (1 μg ml$^{-1}$) was studied, the intensity of fluorescence being maximum and constant between 0.02 and 0.05% of reagent.

The influence of temperature between 15 and 40 °C was investigated, and it was found that the maximum fluorescence was obtained over the temperature range 15–25 °C. For higher temperatures a decrease in $I_F$ values was observed, and both lead - morin - Genapol and lead - morin systems showed strong instability and poor reproducibility. A temperature of 25 °C was chosen for all subsequent experiments.

## Analytical Characteristics

The calibration graph was linear for up to 1 μg ml$^{-1}$ of lead, and a detection limit of 0.06 μg ml$^{-1}$ was found. The relative standard deviation, $s_r$, for 11 replicate analyses of a sample containing 1 μg ml$^{-1}$ of lead was 0.47%.

The effect of 21 ions on the fluorescence of the lead - morin - Genapol system was studied for 0.2 μg ml$^{-1}$ of lead (Table 1). The tolerance in the $I_F$ values was 2%. Ions forming fluorescent complexes with morin strongly interfere, increasing the fluorescence intensity of the system. This has been observed for Al(III) and Be(II) for which the maximum tolerances were found to be 1 and 5 ng ml$^{-1}$, respectively. Sn(II) also interferes, probably owing to the formation of a fluorescent complex with morin. Zn(II) forms a weak fluorescent complex with morin, but in the presence of Genapol PF-20 this system is stabilised, its fluorescence being strongly

increased.[11] For this reason Zn(II) interferes in the Pb - morin - Genapol system; similarly, Zn(II) interferes in the determination of Al(III) with morin in the presence of this non-ionic surfactant.[5]

Low concentrations of Fe(III) and Cu(II) decrease the fluorescence of the system, with tolerance limits of 50 and 25 ng $ml^{-1}$, respectively.

## References


1. Ishibashi, N., and Kina, K., *Anal. Lett.*, 1972, **5**, 637.
2. Sanz Medel, A., and Garcia Alonso, J. I., *Anal. Chim. Acta*, 1984, **165**, 159.
3. Sanz Medel, A., Garcia Alonso, J. I., and Blanco Gonzalez, E., *Anal. Chem.*, 1985, **57**, 1681.
4. Hinze, W. L., Singh, H. N., Baba, Y., and Harvey, N. G., *Trends Anal. Chem.*, 1984, **3**, 193.
5. Medina Escriche, J., de la Guardia Cirugeda, M., and Hernandez Hernandez, F., *Analyst*, 1983, **108**, 1386.
6. Pilipenko, A. T., Vasil'chuk, T. A., and Volkova, A. I., *Zh. Anal. Khim.*, 1983, **38**, 855.
7. Goto, H., *Chem. Zentrabl.*, 1941, **112**, 1068.
8. Gertner, A., Kodrnja, D., Pasivic, D., and Grdinic, V., *Acta Pharm. Jugosl.*, 1971, **21**, 1250.
9. Kopacz, M., *Pr. Nauk. Inst. Chem. Nieorg. Metal Pierwiastkow Rzadkich Politech. Wroclaw.*, 1982, **48**, 102.
10. Mirzaeva, L. K., Makasheva, I. E., and Golovkina, M. T., *Zh. Obshch. Khim.*, 1982, **52**, 1631.
11. Hernandez Hernandez, F., Medina Escriche, J., and Gasco Andreu, M. T., *Talanta*, submitted for publication.


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# Thin-layer Chromatographic Separation and Determination of Dibutylphosphoric Acid in a Mixture of Monobutylphosphoric Acid and Tributyl Phosphate

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A thin-layer chromatographic (TLC) technique using a silica gel - cellullose plate has been developed for the separation of a mixture of monobutylphosphoric acid ($H_2MBP$), dibutylphosphoric acid (HDBP) and tributyl phosphate (TBP) and for the determination of HDBP. The technique involves initial separation on a TLC plate followed by the determination of isolated HDBP by an indirect spectrophotometric method whereby the concentration of HDBP is measured by its ability to reduce the colour intensity of a thorium - thoron complex at 545 nm. Less than 1 μg of $H_2MBP$ and HDBP can be detected on the TLC plate. The method is suitable for the determination of 40–260 μg of HDBP.

**Keywords:** *Dibutylphosphoric acid determination; thin-layer chromatography; spectrophotometry*

In the Purex process, tributyl phosphate (TBP) diluted with a hydrocarbon is used for the separation and extraction of uranium and plutonium from fission products. In the course of the process TBP is known to undergo degradation leading mainly to the formation of dibutylphosphoric acid (HDBP) and, to a lesser extent, monobutylphosphoric acid ($H_2MBP$). Because of various complications that arise in the Purex process owing to the presence of HDBP in TBP streams,[1] TBP is frequently subjected to solvent clean-up using an alkaline wash prior to recycling. It becomes necessary to develop a suitable method for monitoring the concentration of HDBP, in particular, to assess the performance of the clean-up step and to evaluate the quality of TBP in various TBP streams.

Earlier attempts[2–4] have been made with a paper chromatographic technique to assess the contaminants of a TBP - hydrocarbon mixture. Gas - liquid chromatography (GLC) was then considered a potential tool for the determination of HDBP in a mixture.[5–8] However, the limitation of GLC lies in the fact that the sample must be either methylated before analysis or subjected to high-temperature gas chromatographic conditions.[8] Ion chromatography has also been reported as an alternative method for such an analysis.[9] However, the application of thin-layer chromatography (TLC) for studying the quality of such an extractant has been little reported. Nowak *et al.*[10] employed a cellulose - alumina plate for the qualitative and quantitative (radiometric) determination of TBP degradation products using a $^{32}P$-labelled TBP diluent.

This study was undertaken to develop a simple and rapid TLC technique for the qualitative and quantitative determination of HDBP present in a model mixture containing TBP and a small amount of $H_2MBP$.

## Experimental

### Apparatus

*Spectrophotometer.* Absorption measurements were carried out using a Beckman B spectrophotometer with 1-cm cells.

*Thin-layer chromatographic plates.* Silica gel G (E. Merck, FRG) - cellulose (Field Instruments, UK) (1 + 1) plates (10 × 20 cm with 0.25 mm layers) were used. The plates were eluted once with the eluent and activated at 110 °C for 90 min before use.

### Reagents

Unless specified otherwise all solvents and chemicals were of analytical-reagent grade.

Commercial TBP (Monsanto Chemicals, USA), HDBP and $H_2MBP$ (City Chemical Corp., USA) were purified according to the prescribed method.[6,11]

*Solvent system, butanol - ethanol - water - ammonia solution* (25% *m/V*) (50 + 15 + 30 + 5 *by volume*).

*Chromogenic solution.* Solutions of 0.50% *m/V* $NH_4CNS$ in distilled water and 0.50% *m/V* $FeCl_3$ in 1N HCl were prepared and mixed in equal volumes.

*Thorium standard solution,* 443 μg ml$^{-1}$. Thorium stock solution was prepared by dissolving *ca.* 0.5 g of thorium nitrate [$Th(NO_3)_4.4H_2O$] (Indian Rare Earths Ltd., India) in 100 ml of distilled water and standardised against a standard 0.02 M solution of the disodium salt of EDTA with xylenol orange as indicator. Thorium standard solution was obtained by appropriate dilution of this standardised stock solution.

*Xylenol orange solution.* Prepared by dissolving 100 mg of xylenol orange in 100 ml of 66% ethanol.

*Thoron solution,* 0.2% *m/V.* A 0.2-g mass of thoron [2-(2-hydroxy-3,6-disulpho-1-naphthylazo)phenylarsonic acid, disodium salt)] (Fluka, Switzerland) was dissolved in 100 ml of distilled water.

$H_2MBP$ *stock solution,* 10.47% *m/V.* A 10.47-g mass of $H_2MBP$ was accurately weighed into a 100-ml calibrated flask and made up to the mark with benzene. **Caution**—Benzene is highly toxic and appropriate precautions should be taken.

*HDBP stock solution,* 9.88% *m/V.* A 9.88-g mass of HDBP was accurately weighted into a 100-ml calibrated flask and made up to the mark with benzene.

*TBP stock solution,* 5%. A 15-ml volume of dodecane was added to 3 ml of 30% *V/V* TBP.

*Working solutions.* For qualitative study, 100 μl of each of the above stock solutions of $H_2MBP$ and HDBP and 2.0 ml of TBP were placed in a 10-ml calibrated flask and the volume was made up to the mark with benzene, so that a solution containing 1.047 μg μl$^{-1}$ of $H_2MBP$, 0.988 μg μl$^{-1}$ of HDBP and 1% *V/V* of TBP was obtained. For quantitative purposes, 2 ml of each of $H_2MBP$ and HDBP and 1 ml of TBP stock solutions were mixed to yield a model mixture containing 41.9 μg μl$^{-1}$ of $H_2MBP$, 39.5 μg μl$^{-1}$ of HDBP and 1% *V/V* of TBP.

### Procedure

*Qualitative analysis*

Aliquots of sample mixture were spotted on TLC plates using a graduated lambda pipette (10λ, Fischer, USA). The plates were kept in a developing chamber pre-saturated with the above solvent system for 2 h at 23 ± 2 °C. After development, the plates were dried using a hair drier and the whole plates were sprayed with the chromogenic solution. Both $H_2MBP$ and HDBP appeared as white spots against a light red background, while TBP produced a purple spot at room temperature. The sample was also spotted in band form and a clean separation of the components in bands was also achieved.

*Quantitative analysis*

For quantitative analysis a sample mixture, containing 40–320 μg of HDBP, was spotted in quadruplicate and the plates were developed as described under *Qualitative analysis*. After development, the HDBP spots detected by the chromogenic solution were immediately marked with a needle and scraped from the plate with a fine spatula. The HDBP in these scraped layers was analysed by following a spectrophotometric procedure, based on the ability of HDBP to reduce proportionally the colour intensity of a standard thorium - thoron complex. The scraped layers were transferred into a 10-ml calibrated flask, each containing 6 ml of 0.05 M $Na_2CO_3$ solution. The flasks were placed in a water-bath at 60 °C for 5 min and shaken vigorously. After cooling to room temperature, 0.7 ml of 1 N HCl and 200 μl of standard thorium solution were added to each flask and shaken. This was followed by the addition of 200 μl of concentrated HCl and 0.60 ml of thoron solution. The contents of the flasks were made up the mark with distilled water, shaken vigorously and allowed to stand for 20–30 min for full colour development.[11] After centrifuging each flask, the absorbances of the clear supernatant were measured at 545 nm against an experimental blank obtained from uniformly sprayed TLC plates by scraping off a blank area approximately equal to that of the HDBP spots, and subjecting it to the same treatment.

## Results and Discussion

A clear TLC separation of a mixture of $H_2MBP$, HDBP and TBP (with $R_F$ values of 0.35, 0.67 and 0.88, respectively) was achieved. The limit of visual detection of $H_2MBP$ and HDBP was found to be 0.6–0.8 μg. The respective colours of the spots, which faded a few hours after spraying, could be restored on re-spraying with the same reagent.

Quantitative analysis of HDBP in the model mixture of $H_2MBP$ and TBP was carried out for each aliquot (40–320 μg) applied in quadruplicate to thin-layer plates. The standard deviation of individual results of quadruplicate analyses from their means was 5% (based on a 100% recovery). Using these data a linear calibration graph of amount of HDBP *versus* absorbance was obtained (correlation coefficient 0.996).

In order to examine the effect of a specific amount of blank material (sprayed with chromogenic reagent) from the TLC plate on the absorbance of the standard thorium - thoron complex, identical amounts of thorium were complexed with thoron in the presence and absence of blank material. The observed decrease in absorbance at 545 nm, equal to 0.02–0.025 for the standard thorium - thoron complex, in the presence of a requisite amount of blank material is negligible. The reproducibility and linearity of the calibration graph suggest that the method is suitable for the determination of 40–260 μg of HDBP. The range is restricted to 260 μg of HDBP because of the poor reproducibility obtained for 320 μg of HDBP. Although the present spectrophotometric method using the thorium - thoron complex does not permit the determination of HDBP below 40 μg in a TBP mixture, it has the advantage of simplicity, allowing the determination of HDBP without the usual tedious procedure for inorganic phosphate determination. Further, this method can be used to determine HDBP after separation from other contaminants such as $H_2MBP$.

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## References

1. Brodda, B. J., and Heinen, D., *Nucl. Technol.*, 1977, **34**, 428.
2. Cerrai, E., Cessarino, C., and Gadda, F., *Energ. Nucl. (Milan)*, 1957, **4**, 405.
3. Hardy, C. J., and Scragill, D., *J. Inorg. Nucl. Chem.*, 1959, **10**, 323.
4. Shevedov, V. P., and Rosyanov, S. P., *Zh. Anal. Khim.*, 1959, **14**, 507.
5. Hardy, C. J., *J. Chromatogr.*, 1964, **13**, 372.
6. Brignocchi, A., Gasparini, G. M., and Pizzichini, M., National Nuclear Energy Committee, Report No. RT CH1, 1973, **4**, 73.
7. Lee, Y. C., and Ting, G., *Anal. Chim. Acta*, 1979, **106**, 373.
8. Kuo, C.-H., Chang, F.-C., and Yeh, Y.-C., *Analyst*, 1983, **108**, 1380.
9. Lash, R. P., and Hill, C. J., *J. Liq. Chromatogr.*, 1979, **2**, 417.
10. Nowak, M., Nowak, Z., and Rochon, R. A., *Radiochem. Radioanal. Lett.*, 1974, **20**, 47.
11. Ramanujam, A., Gopalkrishnan, V., Gudi, N. M., and Nadkarni, M. N., "Proceedings of the Nuclear Chemistry and Radiochemistry Symposium, Banaras Hindu University, Banaras, India, 1981," Board of Research in Nuclear Sciences, Department of Atomic Energy Bombay, 1983, p. 500.

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# Extraction and Gas Chromatographic Determination of Residual Formaldehyde in Micro-surgical Materials

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A method for the extraction of residual formaldehyde from surgical materials after sterilisation and its gas chromatographic determination as the 2,4-dinitrophenylhydrazone derivative is described. Quantitative determinations are made using 9-cyanoanthracene as an internal standard, instead of anthracene, as reported previously, because this compound is more stable in solution. The sensitivity is of the order of 10 ng $ml^{-1}$ of extracted aldehyde. To measure the released formaldehyde under the conditions closest to those of the human body, because of the purpose of these samples, the formaldehyde was also extracted with a physiological solution (0.9% NaCl), at 37 °C, with and without stirring.

**Keywords:** *Formaldehyde determination; 2,4-dinitrophenylhydrazone; 9-cyanoanthracene; gas chromatography; micro-surgical materials*

Formaldehyde is used in autoclaves working at low temperatures and pressures for the sterilisation of some heat-sensitive surgical devices, previously sealed in their plastic bags. These include probes and catheters of different porosity and chemical composition, used for therapeutic and diagnostic purposes. After sterilisation the samples are repeatedly treated with continuous jets of pressurised air, but a formaldehyde residue remains in the samples.

It is well known that formaldehyde has irritant and allergenic activities so that many countries have fixed the maximum concentrations tolerated in the working environment. These concentrations vary from 10 p.p.m. for Great Britain (1965) to 0.4 p.p.m. for USSR (1979). Few well established rules exist about the formaldehyde released from surgical devices continually used with direct application to the human body. This paper proposes a rapid, accurate and reproducible method for the extraction and determination of residual formaldehyde in sterilised materials that are regularly used in hospitals.

The determination is based on a chromatographic technique, similar to one reported previously,[1,2] for the separation and determination of aldehydes and ketones and also including formaldehyde. In one of these papers the quantitative determination was made using anthracene as an internal standard, although anthracene is not ideal for routine analysis because its concentration in solution decreases with time, owing to dimerisation.[3] Dimerisation takes place at concentrations of about 40 μg $ml^{-1}$ and is enhanced by light. It was found that the concentration of a solution of anthracene in carbon tetrachloride was reduced to half its original value a few hours after preparation. Therefore another standard was selected, whose retention time is close to that of anthracene, but which does not dimerise; 9-cyanoanthracene satisfies these criteria. Although dimerisation occurs, it does so only very slowly, even at concentrations as high as 100–200 μg $ml^{-1}$,[4] and a solution of 9-cyanoanthracene (100 μg $ml^{-1}$) after 5 months is only 5% less concentrated.

The release of formaldehyde from surgical devices depends on several factors, which have been investigated in this work in order to determine the amount of formaldehyde released under conditions close to physiological.

## Experimental

### Reagents

All the reagents were of analytical-reagent grade.

*2,4-Dinitrophenylhydrazine, saturated solution.* Prepared by dissolving 2.4 g of 2,4-dinitrophenylhydrazine (BDH Chemicals) in 100 ml of 2 M hydrochloric acid.

*Formaldehyde standard solution,* 40 μg $ml^{-1}$. Prepared by diluting formaldehyde (Fluka) in doubly distilled water to give a standard solution whose concentration was verified by iodimetric titration.

*9-Cyanoanthracene standard solution,* 100 μg $ml^{-1}$. Prepared by dissolving 9-cyanoanthracene (Carlo Erba) in carbon tetrachloride.

### Gas Chromatographic Determination

The gas chromatograph (GC) used was a Carlo Erba Fractovap Model 2350, equipped with a flame-ionisation detector (FID). The GC glass column was 5% OV-101 on acid-washed, trimethylchlorosilylated, 60–80 mesh Chromosorb W of dimensions 80 cm × 4 mm i.d. The column temperature was 200 °C, the injector and detector temperature 275 °C and nitrogen carrier gas with a flow-rate of 20 ml $min^{-1}$ was used.

Under these conditions the retention time of the 2,4-dinitrophenylhydrazone of formaldehyde was 2.33 min and that of 9-cyanoanthracene was 1.15 min. The volume injected into the GC was 2 μl. The extracts once prepared and derivatised do not require immediate analysis, as they are stable for several days. With the GC method, the sensitivity achieved is of the order of 10 ng $ml^{-1}$ of extracted formaldehyde.

### Extraction of Formaldehyde

The general procedure for the extraction of formaldehyde from the samples was as follows. A piece of each sample of known total area, removed from its plastic bag, which had also been sterilised, was introduced into a closed tube with 2 ml of doubly distilled water for a fixed time. An aliquot of the resulting extract (1 ml) was placed in a 5-ml tube, 200 μl of a saturated solution of 2,4-dinitrophenylhydrazine and 200 μl of carbon tetrachloride containing a known amount of 9-cyanoanthracene (100–200 μg $ml^{-1}$) were added to the tube, which was stirred for 10 min. A 2-μl volume of the carbon tetrachloride phase was injected into the GC.

Two aspects of formaldehyde release were investigated: (A) the maximum concentration of formaldehyde that can be released; and (B) the amount released under physiological conditions.

*Procedure A*

The maximum amount of released formaldehyde was obtained when the samples were kept for 24 h in 2 ml of water, at room temperature, with stirring: 0.10 μg $cm^{-2}$ for PVC,

1.34 μg cm$^{-2}$ for silicone rubbers and 6.10 μg cm$^{-2}$ for porous rubbers. The values so obtained were equal to those found when the extraction was carried out at 37 °C, even if the maximum was achieved in a shorter time in the latter case (6–8 h). These values can be considered to be the total formaldehyde contents.

*Procedure B*

The amount of formaldehyde released under physiological conditions was studied by extracting it at 37 °C in 2 ml of physiological solution (0.9% NaCl) for 2, 4, 7 and 24 h, without stirring.

## Results and Discussion

The methods for the extraction and gas chromatographic determination of formaldehyde, described above, have been applied to two series of 20 samples. The results can be summarised as follows.

The samples sterilised in a cycle with no addition of formaldehyde do not release any formaldehyde, the concentration in the extraction solution being similar to that of a blank.

The concentration of formaldehyde increases with the time of extraction, reaching a plateau after several hours, which strongly depends on the temperature (at room temperature the plateau is achieved after 24 h and at 37 °C after 6–8 h). The magnitude of the plateau value is independent of temperature.

The two parameters that seem to be particularly important are temperature and stirring: the release was significantly increased by stirring or by increasing the temperature. For instance the release is two orders of magnitude higher at 37 °C than at room temperature, for an extraction time of 1 h.

The substitution of doubly distilled water with a physiological solution has no influence in the amount of formaldehyde released: this result holds for all the samples examined, independent of their chemical composition.

The chemical composition of the samples greatly influences the amount of formaldehyde released, which is lower for PVC and silicone rubbers than for porous rubbers. Porous rubber samples, because of their porosity, are able to adsorb an amount of formaldehyde five times higher than PVC and silicone rubbers.

**Table 1.** Release of formaldehyde (μg cm$^{-2}$) using various extraction times for various micro-surgical materials after autoclaving. The relative standard deviation is 2%

| | Amount of formaldehyde extracted/μg cm$^{-2}$ | | | | |
|---|---|---|---|---|---|
| | Procedure A, | Procedure B | | | |
| Sample | 24 h | 2 h | 4 h | 7 h | 24 h |
| PVC | 0.10 | 0.04 | 0.06 | 0.09 | 0.10 |
| Silicone rubbers | 1.34 | 0.55 | 0.96 | 1.21 | 1.34 |
| Porous rubbers | 6.10 | 2.40 | 4.10 | 5.80 | 6.10 |

The amount of formaldehyde released decreases with the time elapsed from the sterilisation of the samples. (This is expected because formaldehyde is a volatile substance.) The decrease is about 50% for porous rubbers and 70% for PVC 2 weeks after the sterilisation, approaching 90% for all the samples examined after 8 months.

The results obtained for three different samples (PVC, silicone rubbers and porous rubbers) using Procedures A and B are given in Table 1. All the samples were autoclaved under identical conditions and for all the samples considered the release was far lower than the level considered toxicologically dangerous.[5]

## References

1. Hoshika, Y., and Takata, Y., *J. Chromatogr.*, 1976, **120**, 379.
2. Kallio, H., Linko, R. R., and Kaitaranta, J., *J. Chromatogr.*, 1972, **65**, 355.
3. Fritzche, J., *J. Prakt. Chem.*, 1867, **101**, 333.
4. Cowan, D. O., and Drisko, R. L., "Elements of Organic Photochemistry," Plenum Press, New York, 1976.
5. Circolare No. 57, June 22nd, 1983, Ministero della Sanità, Direzione Generale Servizi Igiene Pubblica, Divisione V, Rome.

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# Diazotised Sulphanilic Acid as a Spectrophotometric Reagent for the Determination of Trace Amounts of Indole in Aqueous Solution

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A spectrophotometric method for the determination of trace amounts of indole in aqueous solution is described. The method is based on the coupling reaction of the determinand with diazotised sulphanilic acid to form, in an acidic medium, an intense yellow, water-soluble, stable azo dye, which shows maximum absorption at 445 nm. The graph of absorbance *versus* concentration in linear, indicating that Beer's law is obeyed over the range 30–90 μg of indole in a final volume of 25 ml, *i.e.*, 1.2–3.6 p.p.m., with a molar absorptivity of $33.4 \times 10^3$ l mol$^{-1}$ cm$^{-1}$, a sensitivity index of 0.0035 μg cm$^{-2}$, a relative error of 0 to −1.6% and a relative standard deviation of 0.2–1.9%, depending on the determinand concentration. The optimum reaction conditions and interferences from foreign organic compounds have been investigated.

**Keywords:** *Indole determination; aqueous solution; diazotised sulphanilic acid; spectrophotometry*

Indole, a secondary amine, is a natural product of pancreatic digestion, bacterial action and putrefactive decomposition.[1] It occurs naturally, in coal tar and jasmine and nerdi oils, amongst others, and is a product in the manufacture of synthetic indigo.[2,3] The determination of trace amounts of indole is usually carried out using spectrophotometric methods. Reagents used for the spectrophotometric determination of indole are numerous, the most popular being 4-dimethylaminobenzaldehyde.[1] However, this reagent requires a highly acidic medium, the indole should be dissolved in chloroform[1] and sometimes heating and extraction are necessary.[4] These critical conditions might hinder the application of the reagent for the routine determination of indole. The other spectrophotometric methods described involve either reaction with *p*-benzoquinone,[5] a fresh solution of xanthydrol in absolute ethanol,[6] nitrous acid,[7] iron(III) chloride in concentrated hydrochloric acid[8] or β-naphthoquinone sodium monosulphonate.[9] All of these methods have various drawbacks. Therefore, a new method for the trace determination of indole seemed desirable.

To our knowledge, a method based on the formation of an azo dye has not yet been reported. This paper describes such a method based on the coupling reaction of the determined with diazotised sulphanilic acid in an acidic medium. This method has the advantages of simplicity and the complex formed is very stable.

## Experimental

### Apparatus

Spectral measurements were performed on a Shimadzu UV-210 A digital double-beam recording spectrophotometer and absorbance readings were carried out on a Bausch and Lomb Spectronic 710 digital single-beam spectrophotometer using 1-cm silica cells.

### Reagents

All chemicals were of analytical-reagent grade.

*Indole stock solution*, 10 mg ml$^{-1}$. A 0.250-g amount of indole was dissolved in ethanol in a 25-ml calibrated flask and the solution was made up to volume with the same solvent.

*Indole working solution*, 50 μg ml$^{-1}$. A 0.5-ml volume of the indole stock solution was transferred into a 100-ml calibrated flask and the volume was completed to the mark with distilled water.

*Diazotised sulphanilic acid solution*, 50 mM. Prepared by dissolving 0.865 g of sulphanilic acid in about 50 ml of hot distilled water, which was then cooled and transferred quantitatively into a 100-ml calibrated flask containing 1 ml of concentrated hydrochloric acid. This mixture was cooled to about 5 °C using an ice-bath. A 0.350-g mass of sodium nitrite was then added and the mixture was stirred vigorously at intervals over a period of 30 min. Any excess of nitrite was destroyed by the addition of 1% urea solution, and then the volume was completed to the mark with additional, cooled (5 °C), distilled water. This reagent was stable for at least 3 d when kept in a brown bottle in a refrigerator at about 5 °C.

*Hydrochloric acid*, 1 N. Prepared by dilution of concentrated acid.

*Foreign compound solutions*, 1 mg ml$^{-1}$. Prepared by dissolving an amount of the foreign compound in ethanol and completing to volume with distilled water.

### Procedure

To a series of 25-ml calibrated flasks, transfer increasing volumes of indole working solution to cover the range 30–90 μg. Add 2.5 ml of diazotised sulphanilic acid solution and allow the reaction mixture to stand for 20 min. Add 10 ml of 1 N hydrochloric acid and dilute to the mark with distilled water. Measure the absorbance at 445 nm against a reagent blank, prepared in the same way but containing no indole, using 1-cm cells. The colour is stable for more than 60 min. A linear calibration graph is obtained indicating that Beer's law is obeyed over the concentration range 30–90 μg per 25 ml, *i.e.*, 1.2–3.6 p.p.m. The conditional molar absorptivity of the yellow system (referred to indole) in the region of least photometric error, and at the wavelength of maximum absorption, is found to be $33.4 \times 10^3$ l mol$^{-1}$ cm$^{-1}$.

## Results and Discussion

### Absorption Spectra

When a dilute aqueous solution of indole and diazotised sulphanilic acid reagent solution were mixed in an acidic medium, a yellow azo dye was formed. This intensely coloured compound showed maximum absorption at 445 nm in contrast to the reagent blank, which showed no absorption over the region scanned (380–600 nm). Fig. 1 shows the spectra of the azo dye and of the reagent blank. The maximum absorption at 445 nm, characteristic of the azo dye, was utilised in all subsequent experiments.

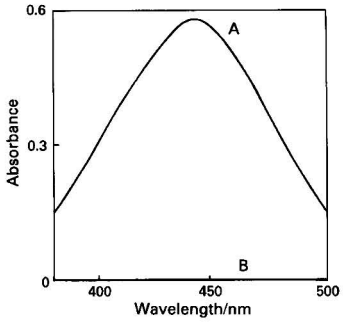


**Fig. 1.** Absorption spectra of A, 50 μg of indole, treated as described under Procedure and measured against a reagent blank; and B, reagent blank measured against distilled water

**Table 1.** Accuracy and precision of the proposed method

| Amount of indole taken/μg | Relative error, % | Relative standard deviation,* % |
|---|---|---|
| 30.0 | 0.0 | 0.2 |
| 60.0 | −1.6 | 1.9 |
| 90.0 | −1.4 | 1.9 |

* Calculated from five determinations.

**Table 2.** Effect of some organic compounds on the determination of 50 μg of indole

| Interferent | Amount added/ μg | Interference, % |
|---|---|---|
| 2-Aminopyrimidine | 100 | +2.0 |
| Cytosine | 100 | +3.0 |
| Histidine | 100 | −2.1 |
| 4-Hydroxyproline | 100 | +5.0 |
| Imidazole | 100 | +3.3 |
| Indole-3-acetic acid | 100 | +4.8 |
| Nicotinic acid | 100 | −4.7 |
| 1,10-Phenanthroline (monohydrate) | 100 | +5.5 |
| Proline | 100 | −0.9 |
| Pyrazole | 100 | +1.7 |
| Pyrrole-2-carboxylic acid | 100 | −0.1 |
| Tryptophan | 100 | −1.9 |
| Uracil | 100 | +0.8 |

### Effect of Diazotised Reagent

In order to ascertain the optimum spectrophotometric conditions,[10] several diazotised reagents were investigated to establish the most useful reagent for the determination of indole. Of the diazotised reagents tested, including diazotised orthanilic acid, diazotised 4-aminoacetophenone, diazotised 4-nitroaniline and diazotised sulphanilic acid, diazotised sulphanilic acid gave the most satisfactory results. This reagent has not been used previously for the determination of indole, although it is a commonly used reagent.[11] Diazotised sulphanilic acid formed a water-soluble azo dye with indole under the experimental conditions, thus avoiding the time-consuming extraction process, in contrast to diazotised 4-nitroaniline, which, although giving a more sensitive reaction with indole, formed a water-insoluble azo dye. Therefore, diazotised sulphanilic acid was studied further. Increasing the diazotised sulphanilic acid concentration led to an increase in the colour intensity; 2.5 ml of 50 mM diazotised sulphanilic acid solution provided the optimum absorbance. Finally, the diazotisation of sulphanilic acid was carried out in different acids to evaluate the best acid. The experimental data revealed hydrochloric acid to be the most suitable of the commonly known acids.

### Effect of Acids

The preliminary results indicated that the presence of an acid in the reaction mixture is essential for developing a more intense colour. In this respect, acetic, sulphuric, phosphoric and hydrochloric acids were examined. It was found that the best results were obtained with hydrochloric, sulphuric and phosphoric acids; acetic acid produced only a pale colour. Hydrochloric acid was chosen, and 10 ml of 1 N solution were added after the diazotised reagent, producing the optimum absorbance.

### Order of Addition of Reagents

To obtain the optimum results, the order of addition of reagents should be followed as given under Procedure, otherwise a loss in colour intensity is observed.

### Development Time and Stability Period

In spite of the rapid colour development, the reaction mixture was allowed to stand for 20 min before addition of the acid and appropriate dilution, in order to attain full colour development. Afterwards the colour remained stable for more than 1 h, after which a slight decrease was observed. The above stability period was sufficient to allow several measurements to be performed sequentially.

### Accuracy and Precision

To determine the accuracy and precision of the method, indole was determined at three different concentrations. The results are shown in Table 1 and indicate that a satisfactory precision and accuracy could be attained using this method.

### Interferences

To demonstrate the selectivity of the method, the interfering effects of some organic compounds were examined by carrying out the determination of 50 μg of indole in the presence of each of the interferents using the recommended procedure. The results obtained are given in Table 2. Diphenylamine and pyrrole interfered seriously.

## Conclusion

A simple and sensitive spectrophotometric method for the determination of trace amounts of indole in solution has been devised, based on coupling with diazotised sulphanilic acid. The proposed method requires neither temperature control nor solvent extraction.

## References

1. Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," Volume 4, Third Edition, Van Nostrand, New York, 1954, pp. 288–289.
2. Brady, G. S., and Clauser, H. R., "Materials Handbook," Eleventh Edition, McGraw-Hill, New York, 1977, p. 390.
3. Morrison, R. T., and Boyd, R. N., "Organic Chemistry," Third Edition, Allyn and Bacon, Boston, 1973, p. 1010.
4. Lesiak, T., *Chem. Anal. (Warsaw)*, 1965, **10**, 223.
5. Pesez, M., and Bartos, J., *Ann. Pharm. Fr.*, 1965, **23**, 783.
6. Fearon, W. R., and Drum, J. A., *Sci. Proc. R. Dublin Soc.*, 1951, **25**, 295.
7. Grönwall, M., *Skand. Arch. Physiol.*, 1938, **78**, 139.
8. Sanders, J. A., and May, C. E., *Biochem. Bull.*, 1913, **2**, 373.
9. Herter, C. A., and Foster, M. L., *J. Biol. Chem.*, 1905–6, **1**, 257.
10. Sulaiman, B., and Bashir, W. A., *Analyst*, 1984, **109**, 1409.
11. Whitlock, L. R., Siggia, S., and Smola, J. E., *Anal. Chem.*, 1972, **44**, 532.

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# Selective Spectrophotometric Kinetic Determination of Cobalt with *o*-Hydroxyphenylthiourea*

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A simple kinetic method has been developed based on the autoxidation of *o*-hydroxyphenylthiourea to disulphide, which is catalysed by trace amounts of cobalt. The reaction is monitored spectrophotometrically at pH 8.0 and cobalt is determined effectively in the range 5–50 ng ml$^{-1}$. This method has been used to determine trace amounts of cobalt present in certain plant materials such as carrot roots and corn grains, in which it plays a vital role in different physiological activities.

**Keywords:** *Cobalt determination; spectrophotometry; autoxidation; o-hydroxyphenylthiourea*

A simple and selective catalytic method has been developed for the determination of nanogram amounts of cobalt(II) based on the autoxidation of *o*-hydroxyphenylthiourea (OHPTU), a reagent that has been used previously for the determination of trace amounts of copper(II)[1] and manganese(II).[2] The reaction was followed spectrophotometrically at 416 nm and the selectivity of the method was improved by the use of masking agents. The method was applied to the determination of cobalt in foodstuffs.

## Experimental

### Apparatus

A Toshniwal Model RL04/01 spectrophotometer and an Elico Model PH820A pH meter were used.

### Reagents

The reagents used were of analytical-reagent grade unless otherwise specified.

*o-Hydroxyphenylthiourea (OHPTU).* Prepared by the standard procedure.[3] Its purity was confirmed from melting-point determinations (161 °C) and elemental analysis, which gave the following results: theoretical, C 50.04, H 4.80, N 16.66 and S 19.04; found (CDRI, India), C 50.15, H 5.05, N 16.10 and S 18.85%.

*Reagent solution,* 1 mg ml$^{-1}$. Prepared freshly in distilled ethanol before use.

*Cobalt(II) stock solution,* 1 mg ml$^{-1}$. Prepared by dissolving cobalt(II) chloride (BDH Chemicals) in doubly distilled water. This solution was standardised titrimetrically.[4]

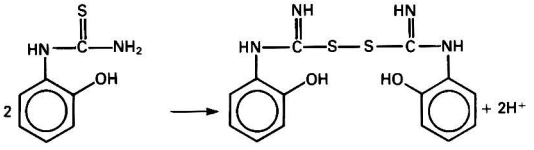
*Buffer solution, pH* 8.0. Prepared from 0.05 M disodium tetraborohydrate and 0.05 M sodium hydroxide solutions.

### Recommended Procedure

To a solution containing 125–1400 ng of cobalt, add 7 ml of buffer solution (pH 8.0), 1 ml of 0.025 M pyridine solution (as a promoter) and 4.0 ml of reagent solution and make up to 25 ml with doubly distilled water. Measure the absorbance at 416 nm and plot the absorbance against time.

## Results and Discussion

OHPTU undergoes autoxidation to form a yellow disulphide, *N,N'*-bis(*o*-hydroxyphenyl)-1,1'-dithiobisformamidine:



This reaction is very slow, but is sharply increased by the addition of trace amounts of cobalt(II). Increasing the concentration of cobalt increases the rate of the catalysed oxidation and it has been found that the rate of the reaction is proportional to the concentration of cobalt. The $\lambda_{max.}$ values of OHPTU and its oxidised product are 280 and 416 nm, respectively, and hence an excess of reagent does not affect the absorbance due to the formation of disulphide.

In this study cobalt was determined using the tangent method in which tan θ values (or slopes) of graphs of absorbance *versus* time were determined for various amounts of cobalt (Fig. 1) and a final calibration graph was constructed by plotting the value of tan θ (or slope) against concentration of cobalt (Fig. 2). The calibration graph was linear in the range 5–50 ng ml$^{-1}$ of cobalt. The fixed-time method was also used for the kinetic determination of cobalt. In this method, the absorbance of the solution was measured after allowing the reaction to proceed for a fixed time and the calibration graph constructed by plotting a graph of absorbance against concentration (Fig. 2).

The determination of cobalt was possible in the pH range 7.5–8.5. Below pH 7.5, the catalysed autoxidation rate was slow and above pH 8.5, the uncatalysed reaction was sufficiently fast. Hence, a pH of 8.0 was chosen for further studies. A change of 0.5 pH unit was tolerable but an appreciable change of pH during the reaction was prevented

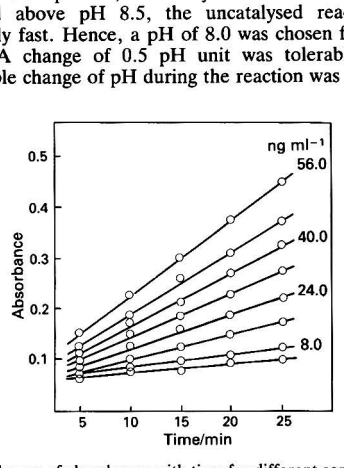



**Fig. 1.** Change of absorbance with time for different concentrations of cobalt

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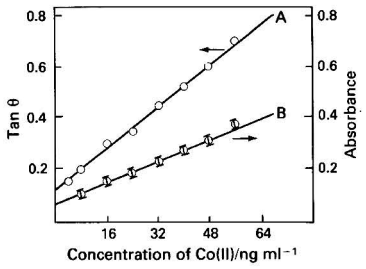


**Fig. 2.** Calibration graphs for determination of cobalt: A, tangent method; and B, fixed-time method

**Table 1.** Determination of cobalt in plant materials

| Sample | Cobalt found: Present method using OHPTU*/ ng g$^{-1}$ | Cobalt found: Standard method using nitroso-R salt/ ng g$^{-1}$ |
|---|---|---|
| Carrot root (*Daucus carota* var. *sativa*) | 22.0 ± 0.3 | 22.5 ± 0.5 |
| Corn grain (*Zea mays*) | 10.5 ± 0.2 | 10.5 ± 0.3 |

* Average of five determinations.

by using a buffer. Increasing the temperature did not have a significant effect on the reaction rate. The addition of 1 ml of 0.025 M pyridine as a promoter increased the sensitivity of the method; an excess of promoter did not affect the reaction rate provided that the pH was kept within the given range. The rate increased with increase in the concentration of the reagent; however, 4.0 mg of the reagent was sufficient to determine up to 50 ng of cobalt. The method has a good reproducibility. Even in the fixed-time method, the relative standard deviation for 40 ng of cobalt was 0.37%. In Fig. 2B, standard deviations of the absorbances are indicated on the points.

To study the effect of dissolved oxygen, an alcoholic solution (water - ethanol, 80 + 20 *V/V*) was heated nearly to boiling and cooled by passing hydrogen through it. To this solution, solid OHPTU was added. A blank experiment was conducted similarly and, after an interval of 30 min, the absorbance was measured at 416 nm. There was no difference in the absorbance values.

### Interference Studies

The effect of foreign ions on the determination of 40 ng of cobalt was studied by the fixed-time method. A change in absorbance of ±0.025 was considered to be the tolerance limit for interference. Metal ions such $Al^{3+}$, $Cd^{2+}$, $Ni^{2+}$, $Mg^{2+}$, $Se^{4+}$, $Te^{4+}$, $Li^{+}$, $Ca^{2+}$, $Ba^{2+}$, $Sr^{2+}$, $Sn^{2+}$, $Be^{2+}$, $UO_2^{2+}$, $Sb^{2+}$, $Tl^{3+}$, $La^{3+}$, $Zr^{4+}$ and $Zn^{2+}$ did not interfere even when present in up to a 2000-fold excess by mass. $Mo^{6+}$ and $W^{6+}$ up to a 65-fold excess and $Cr^{6+}$ and $Ce^{4+}$ in up to a 40-fold excess did not interfere in the reaction. $Cu^{2+}$ and $Pt^{4+}$ interfered by enhancing the absorbance and $Pb^{2+}$, $Pd^{2+}$, $Hg^{2+}$ and $Ag^{+}$ interfered by producing turbidity. The interference of iron and manganese was eliminated by adding fluoride and oxalate, respectively. Common anions such as fluoride, chloride, sulphate, nitrate and oxalate had no effect on the reaction rate. Phosphate interfered in the determination when present in a large excess (1000-fold).

### Determination of Cobalt in Foodstuffs

Table 1 shows the results for the determination of cobalt in foodstuffs such as carrot root and corn grain. The results obtained were compared with those obtained with a standard procedure using nitroso-R salt,[5] for the same samples. The results show good agreement between the two methods.

## Conclusion

The method described provides a reliable and simple means of determining trace amounts of cobalt by spectrophotometry. The sensitivity of this method is superior to the standard method based on nitroso-R salt and, because it is free from interference, is a useful method for the precise determination of trace amounts of cobalt in biological materials.

Further, it is more sensitive than the procedures developed by Prik and Orlova,[6,7] Costache and co-workers,[8-10] Igov *et al.*[11] and Alexiev and Angelova[12] and is fairly selective.

One of us (S. J. R.) is grateful to UGC, New Delhi, for financial assistance.

## References

1. Rao, S. J., Reddy, G. S., and Reddy, Y. K., *Indian J. Chem.*, in the press.
2. Rao, S. J., Reddy, G. S., and Reddy, Y. K., *Proc. Indian Acad. Sci.*, in the press.
3. "Beilsteins Handbuch der Organischen Chemie," Volume XIII, Edwards Brothers, Ann Arbor, MI, 1943, p. 375.
4. Vogel, A. I., "A Text Book of Quantitative Inorganic Analysis," Third Edition, Longman, London, 1962, p. 443.
5. Vogel, A. I., "A Text Book of Quantitative Inorganic Analysis," Third Edition, Longman, London, 1962, p. 795.
6. Prik, G. A., and Orlova, M. N., *Uch. Zap. Vladimir. Gos. Pedagog. Inst.*, 1970, **22**, 48.
7. Greinke, R. A., and Mark, H. B., Jr., *Anal. Chem.*, 1972, **44**, 295R.
8. Costache, D., and Popa, G., *Rev. Roum. Chim.*, 1971, **16**, 761.
9. Greinke, R. A., and Mark, H. B., Jr., *Anal. Chem.*, 1974, **46**, 413R.
10. Costache, D., and Junie, P., *Rev. Roum. Chim.*, 1971, **16**, 597.
11. Igov, R. P., Jaredic, M. D., and Pecev, T. G., *Bull. Soc. Chim. Beograd.*, 1980, **45**, 365.
12. Alexiev, A. A., and Angelova, M. G., *Mikrochim. Acta*, 1980, **II**, 197.

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# Spectrophotometric Determination of Selenium(IV) with Potassium Butyl Xanthate

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A simple and convenient method for the spectrophotometric determination of selenium(IV) has been developed using potassium butyl xanthate as an analytical reagent. The optimum concentration range evaluated by Ringbom's method was found to be 5–12 p.p.m. and the optimum pH range 2.0–4.7. The composition of the complex was studied by the molar ratio method and Job's method of continuous variations. The effect of foreign ions on the determination was also studied.

**Keywords:** *Selenium(IV) determination: potassium butyl xanthate; visible spectrophotometry*

The methods recommended for the determination of selenium, *e.g.*, iodimetric titration with sodium thiosulphate or ascorbic acid reduction,[1] have limits of detection of *ca.* 50 mg and 50 μg, respectively. The American Public Health Association Standard Methods[2] recommend the diaminobenzidine spectrophotometric method with or without distillation. Both are long processes and both diaminochryzin[3] and thioacetamide[4] require reaction at high temperatures. Singh *et al.*[5] separated selenium(IV) and tellurium(IV) morpholine-4-carbodithioates, quantitatively, using TLC and visible spectrophotometry.

In this work potassium butyl xanthate (KBX) was used as a spectrophotometric reagent for the quantitative determination of selenium(IV). The selenium butyl xanthate has a high degree of stability.

## Experimental

### Apparatus

A Bausch and Lomb Spectronic 20 spectrophotometer was used for the absorbance measurements and a Philips PP 9040 pH meter with glass - calomel electrodes was used for the measurement of pH.

### Synthesis of the Reagent

Potassium butyl xanthate was prepared by dissolving potassium hydroxide in distilled water, adding benzene followed by butanol with constant stirring and finally adding carbon disulphide very slowly, maintaining the temperature at *ca.* 25 °C. (**Caution**—Benzene is highly toxic and appropriate precautions should be taken.) The proportions of KOH solution, butanol and carbon disulphide were 1 + 1 + 1. The solid product was filtered, washed with diethyl ether and dried under vacuum.

### Reagents and Solutions

*Potassium butyl xanthate stock solution*, 0.1882% *m/V*. Prepared by dissolving a known mass of the reagent in distilled water.

*Selenium dioxide stock solution*, 0.02 M. Prepared by dissolving the appropriate amount of selenium dioxide (Central Drug House, New Delhi), in doubly distilled water and standardising iodimetrically.

*Acetic acid - sodium acetate buffer solution, pH* 4.0.

### Determination of Selenium

To the selenium dioxide solution containing up to 221 p.p.m. of Se(IV) were added 0.5 ml of acetate buffer (pH 4.0) and 1.5 ml of 0.188% *m/V* potassium butyl xanthate solution, in a 25-ml glass-stoppered tube. The solutions were mixed and allowed to stand for 40 min for the reaction to go to completion. The selenium butyl xanthate so formed was then shaken with 8 ml of carbon tetrachloride for 2 min to extract the complex. The two phases were separated and the absorbance of the organic phase was measured at 395 nm against a blank obtained by extraction of the reagent containing no selenium.

### Composition of the Complex

When an aqueous solution of KBX and Se(IV) interacted, a yellow oily chelate was formed. The reaction was complete in 35 min and the selenium butyl xanthate formed was completely extractable in carbon tetrachloride. Maximum absorbance was attained with an eight-fold excess of the reagent. The complex absorbed strongly at 395 nm, the wavelength chosen for further studies.

*Molar ratio method*

Equimolar solutions of selenium dioxide and reagent of 0.002 and 0.004 M were used. A series of solutions was prepared, keeping the concentration of selenium ions constant (1 ml of 0.002 and 0.004 M) while varying the concentration of KBX (1–9 ml of 0.002 and 0.004 M) and the pH values of the solutions were adjusted to 4.0 with acetate buffer. The mixtures were allowed to stand for 40 min for completion of the reaction. The selenium butyl xanthate was then extracted with 8 ml of carbon tetrachloride and the absorbance was measured against a blank obtained by extraction of the reagent containing no selenium. The absorbance increased up to an eight-fold excess of the reagent but increased steeply in the presence of up to a four-fold excess of reagent[6] (Fig. 1).

*Jobs method of continuous variations*[7,8]

Equimolar solutions of selenium dioxide and KBX were mixed in complementary proportions to a fixed total volume. The procedure adopted for the preparation of the solutions was the same as described earlier. The plot of absorbance

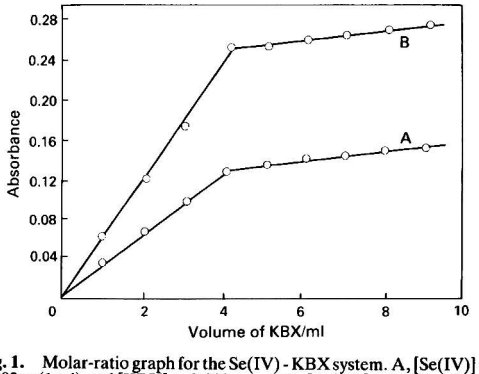


**Fig. 1.** Molar-ratio graph for the Se(IV) - KBX system. A, [Se(IV)] = 0.002 M (1 ml) and [KBX] = 0.002 M; and B, [Se(IV)] = 0.004 M (1 ml) and [KBX] = 0.004 M

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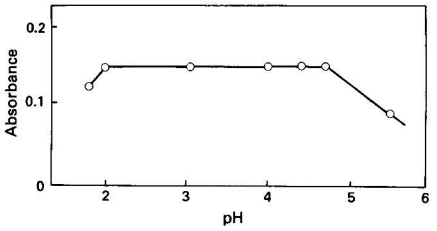


**Fig. 2.** Effect of pH on the absorbance of Se(IV) - KBX

*versus* $\{[M]^{4+}/([M]^{4+} + [R])\}$ produced a graph that indicated the formation of a chelate having a selenium to reagent ratio of 1:4.

*Effect of pH*

The absorption spectra of selenium butyl xanthate at different pH values between 1.8 and 5.5 indicated that complex formation starts at pH 1.8 and produces a maximum at pH 2.0–4.7. Above pH 5.5 the solution becomes red, possibly owing to the separation of selenium from the complex (Fig. 2).

Vosburgh and Cooper's method[9] indicated the existence of only one complex at 395 nm, which was studied at pH 4.0.

### Choice of Solvents

For the extraction of yellow oily selenium butyl xanthate, carbon tetrachloride, chloroform, benzene, 1,4-dioxane, pyridine, methanol, ethanol, butanol, dodecylene and hexane solvents were tried.

The selenium butyl xanthate was extractable in carbon tetrachloride, benzene and chloroform but the maximum absorbance was obtained in carbon tetrachloride. The colour of the complex became red with methanol, ethanol, butanol and acetone, which may be due to the separation of elemental selenium from the complex. The selenium butyl xanthate was soluble in 1,4-dioxane and pyridine.

### Linearity and Sensitivity

Under experimental conditions it was observed that Beer's law was obeyed from 5 to 17 p.p.m. of Se(IV), a linear calibration graph being obtained, which passed through the origin. The molar absorptivity calculated from Beer's law is $6.1 \times 10^2$ l mol$^{-1}$ cm$^{-1}$ at 395 nm and Sandell's sensitivity[10] of the colour reaction is 0.1294 μg cm$^{-2}$.

The optimum concentration for the effective spectrophotometric determination of Se(IV), evaluated by Ringbom's method[11,12] was found to be 5.0–12 p.p.m.

### Effect of Foreign Ions

The interference of foreign ions on the determination of 15.79 p.p.m. of selenium was investigated. Fe(II), Sn(II), Pb(II) and Zn(II) form complexes with the reagent, which are not extractable in carbon tetrachloride and therefore do not interfere.

The interference of Co was avoided by masking with ethylenediaminetetraacetic acid (EDTA).

The effect of foreign ions and their interference limits are shown in Table 1.

## Results and Discussion

Potassium butyl xanthate formed a yellow oily complex with Se(IV) in an acidic medium. The reaction was slow, requiring 30–40 min for full complex formation. The absorbance of the complex in carbon tetrachloride remained stable for at least 24 h, and thereafter the absorbance changed. The maximum absorbance was attained when the reagent was in an eight-fold excess of selenium.

**Table 1.** Effect of foreign ions on the determination of 15.79 p.p.m. of Se(IV) with KBX

| Foreign ion | Concentration, p.p.m. | Se(IV) found/μg | Error, % |
|---|---|---|---|
| $Co^{2+}$ | 9 | 15.79 | Nil |
| $Cr^{3+}$ | 37 | 15.49 | −1.9% |
| $Mg^{2+}$ | 15 | 15.79 | Nil |
| $Fe^{2+}$ | 34 | 15.79 | Nil |
| $As^{3+}$ | 18 | 15.79 | Nil |
| $Ba^{2+}$ | 17 | 15.49 | −1.9% |
| $Fe^{3+}$ | 34 | 15.65 | −0.9% |
| $Sn^{2+}$ | 13 | 15.28 | −3.2% |
| $Ti^{4+}$ | 30 | 15.62 | −1.1% |
| $Zn^{2+}$ | 8 | 15.11 | −4.3% |
| $PO_4^{3-}$ | 50 | 15.79 | Nil |
| $NO_2^-$ | 31 | 15.79 | Nil |
| $S_2O_3^{2-}$ | 50 | 15.98 | +1.2% |
| $Cl^-$ | 50 | 15.49 | −1.9% |
| $Br^-$ | 50 | 15.49 | −1.9% |
| $I^-$ | 59 | 15.49 | −1.9% |
| $SO_4^{2-}$ | 50 | 15.49 | −1.9% |
| $CN^-$ | 32 | 15.79 | Nil |
| $CH_3COO^-$ | 50 | 15.65 | −0.9% |
| $MoO_4^{2-}$ | 18 | 15.28 | −3.2% |
| EDTA | 47 | 15.49 | −1.9% |

**Table 2.** Determination of selenium(IV)

| Selenium(IV) taken, p.p.m. | Selenium(IV) found in complex, p.p.m. | Deviation, % |
|---|---|---|
| 7.89 | 7.96 | +0.85 |
| 9.86 | 9.88 | +0.12 |
| 11.80 | 11.90 | +0.85 |
| 15.79 | 15.84 | +0.32 |

When the complex was subjected to paper chromatography in different organic solvents (light petroleum, carbon tetrachloride, chloroform) only a single spot developed on the paper, indicating the formation of one complex. The colour of the spot remained unchanged in different solvents but after 12 h the colour of the spot changed from yellow to red, possibly owing to the reduction of the complex to selenium.

### Determination of Selenium

The results are given in Table 2 for the determination of selenium at various levels using the proposed procedure.

## References

1. Saren, R. N., and Pandey, S. P., *J. Inst. Chem.*, 1983, **55**, 205.
2. American Public Health Association, American Water Works Association and Water Pollution Control Federation, "Standard Methods for the Examination of Water and Wastewater," Thirteenth Edition, American Public Health Association, New York, 1971.
3. Brown, R. S., *Anal. Chim. Acta*, 1975, **74**, 441.
4. Futekov, L., and Shocherev, M. G., *Tudy Flodiv, Univ.*, 1972, **10**, 81.
5. Singh, N., Rastogi, K., Kumav, R., and Srivastava, T. N., *Analyst*, 1981, **106**, 599.
6. Yoe, J. H., and Jones, A. L., *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 111.
7. Job, P., *Am. Chem.*, 1928, **9**, 113.
8. Irving, H., and Pierce, I. B., *J. Chem. Soc.*, 1959, **5**, 256.
9. Vosburgh, W. C., and Cooper, G. R., *J. Am. Chem. Soc.*, 1941, **63**, 437.
10. Sandell, E. B., "Colorimetric Determination of Trace Metals," Interscience, New York, 1959.
11. Ringbom, A., *Z. Anal. Chem.*, 1939, **115**, 332.
12. Ayres, G. H., *Anal. Chem.*, 1949, **21**, 652.

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# Oxidative Amperometric Flow Injection Determination of Oxalate at an Electrochemically Pre-treated Glassy Carbon Electrode

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An effective electrochemical pre-treatment of a newly polished glassy carbon electrode has been shown to be essential if sensitive and reproducible signals are to be obtained in the oxidative determination of oxalate. Pre-treatment at +1.75 V for 10 min and then at −1.0 V for 1 min in the 0.05 M phosphate buffer (pH 7.0) used as an eluent for the determination was shown to be effective in producing a well shaped hydrodynamic voltammogram, maximum signal size and improved precision at the measurement potential used (1.5 V). Calibration graphs were rectilinear for up to 100 μg ml$^{-1}$ of oxalic acid dihydrate.

**Keywords:** *Flow injection analysis; oxalate determination; glassy carbon electrode; electrochemical pre-treatment; amperometric detection*

The electrochemical pre-treatment of glassy carbon electrode surfaces has been shown to improve electrode performance in many analytical applications by increasing the electron transfer rate and producing a less irreversible electrode reaction.[1-9] Better shaped and more reproducible voltammograms are obtained. Treatment is believed to result in an increase in the number of surface quinone and other groups, which catalyse the oxidation or reduction of the determinand. Despite this previous work, electrochemical pre-treatment of glassy carbon electrodes that are to be used in electrochemical detector cells in HPLC systems does not appear to be practised widely. This must be partly due to the still limited information on applications where electrochemical pre-treatment has been shown to be advantageous, and further work is required to investigate the wider applications and the durability of such pre-treatments. In many HPLC applications of electrochemical detection currently in use, seemingly good sensitivity and detection limits are obtained simply by using newly polished glassy carbon electrodes. In examples where irreversible electrode reactions are being used, however, it may subsequently be found that sensitivities, detection limits and precision can be improved by electrochemical pre-treatment.

The sometimes marked effect of electrochemical pre-treatment of glassy carbon electrodes was appreciated fully in this laboratory when an oxidative method of determining sulphite was being developed.[8] In 0.05 M sodium carbonate solution chloride was found to catalyse the oxidation and to increase the size of the signal at a glassy carbon electrode that had been newly polished. More importantly, in the absence of chloride the sulphite signals were observed to become increasingly large when the glassy carbon electrode was used for some time without being repolished. This seemed to indicate that some kind of activation of the electrode surface was occurring. With the use of Engstrom's pre-treatment procedure,[5,7] which involves subjecting the electrode to a period of time at a high positive potential (e.g. +1.5 V) and then at a negative potential, well shaped FIA hydrodynamic voltammograms were obtained with a seven-fold increase in signal size at the measurement potential. Chloride no longer affected the oxidation signal and even more importantly the signals were now highly reproducible. For systems such as the sulphite one electrochemical pre-treatment appears to be essential if reproducible signals are to be obtained. EDTA was found to interfere in the determination of sulphite and indeed could be determined by a similar method using an electrochemically pre-treated glassy carbon electrode.[9]

At this time interest was being shown in these laboratories in the determination of oxalate in urine samples. Clinical methods currently in use are apparently time consuming. A method has been published[10] in which oxalic acid in urine is separated as calcium oxalate, which after being redissolved is injected on to an HPLC column containing a strong cation exchanger. The eluent contains a quaternary ammonium salt and an ion pair mechanism is suggested for the retention of the oxalate on the column. An amperometric detector with a wax-impregnated graphite working electrode is used. In this work a study has been made of the determination of oxalate using flow injection analysis with amperometric detection; the effect of electrochemical pre-treatment of a glassy carbon electrode on the signals obtained with oxalate is reported here.

## Experimental

A single-channel flow injection system was used.[11] Eluent (pH 7.0 phosphate buffer in the final recommended procedure) was pumped through the system by means of an Ismatec Mini-S pump. Injections were made with a Rheodyne low-pressure sample injection valve (5020) fitted with a 75-μl sample loop and connected to a laboratory-built amperometric detector by means of 1 m of 0.58 mm bore Teflon tubing. The amperometric detector,[11] which holds a Metrohm glassy carbon electrode in the wall-jet configuration, is used partly immersed in electrolyte of the same composition as the eluent. The platinum counter and saturated calomel reference electrodes are placed in this electrolyte to complete the three-electrode system. The potential of the glassy carbon electrode was maintained at the required potential by means of a PAR 174A polarographic analyser (Princeton Applied Research). Signals were recorded on a Linseis L650 $y$ - $t$ recorder.

### Reagents

*Standard oxalic acid dihydrate solution*, 0.01 M. Dissolve 0.315 g of oxalic acid dihydrate in water and dilute to 250 ml. Prepare less concentrated solutions by dilution.

*Phosphate buffer solution*, pH 7.0. Dissolve 7.80 g of sodium dihydrogen phosphate in 500 ml of water, add 0.1 M sodium hydroxide solution to bring the pH to 7.0 and dilute to 1 l with water.

## Results

Preliminary studies were made with a glassy carbon electrode that had not been electrochemically pre-treated. Linear-sweep voltammograms were obtained for a $10^{-3}$ M oxalic acid solution in Britton - Robinson buffer. A well formed peak, which was largely independent of pH, was observed at about 1.2 V for buffer solutions of pH 1.8–7.0. This peak was not present in pH 8.3 Britton - Robinson buffer. Later in these preliminary studies it was observed that signals obtained in a pH 7.0 phosphate buffer were slightly larger and occurred at a slightly less positive potential than those obtained in the Britton - Robinson buffers. For this reason a 0.05 M pH 7 phosphate buffer solution was used as the eluent in the flow injection experiments. At flow-rates above 5.8 ml $min^{-1}$ the signal obtained at +1.2 V (the measurement potential initially used) using the same volume of the same standard oxalate solution did not increase markedly and a flow-rate of 6.0 ml $min^{-1}$ was adopted subsequently. At this stage it was observed that after repolishing the electrode the size of the signal obtained was dependent on the length of time the electrode had been used at 1.2 V in the eluent stream before the particular injection was made. For example, in one set of experiments the signal obtained with an electrode immediately after it was polished was 0.63 μA but the size of the signal grew steadily with the length of time the electrode was used at +1.2 V and the signal reached a value of 2.03 μA after 25 min of use. Clearly, some form of electrochemical activation process is occurring during this time.

For satisfactory operation of the electrode for the determination of oxalate it is clear that adequate electrochemical pre-treatment of the electrode must be carried out in advance of making analytical measurements if reproducible signals and good accuracy are to be assured. The effects of 5-min electrochemical pre-treatments at various positive potentials between 1.2 and 2.0 V followed by pre-treatment at −1.0 V for 1 min were studied. The signal was seen to increase with a pre-treatment potential of up to 1.4 V but there was only a small increase in the signal at potentials above this. A potential of 1.75 V was chosen for use subsequently and it was found that a completely steady signal was obtained using a 10-min pre-treatment at this potential: no increase in signal size was obtained for pre-treatment times greater than this.

A hydrodynamic voltammogram for the determination of oxalic acid at a glassy carbon electrode pre-treated in this way is shown in Fig. 1; clearly the voltammogram is very well shaped and the use of this electrode was found to give highly reproducible signals. A hydrodynamic voltammogram for the determination of oxalic acid at a newly polished glassy carbon electrode obtained under identical conditions is also given in Fig. 1 for comparison. The improvement in the quality of the signal after electrochemical pre-treatment is apparent. In subsequent work at the pre-treated electrode the detection potential was changed to +1.5 V so that measurements were made on the plateau of the hydrodynamic voltammogram. Typical signals obtained in the range 1–63 μg $ml^{-1}$ of oxalic acid are given in Fig. 2. The calibration graphs show good rectilinearity up to 100 μg $ml^{-1}$ but loss of rectilinearity occurs above this concentration; the coefficient of variation at oxalic acid concentrations from 1 to 100 μg $ml^{-1}$ was found to be typically <1% for ten injections.

## Discussion

The brief study described here has shown that the exact condition of the surface of a glassy carbon electrode that is to be used for the determination of oxalate by flow injection analysis with amperometric detection is important if meaningful, precise and accurate results are to be obtained. It is likely that this is also the case in applying glassy carbon electrodes in amperometric detectors for use in the determination of oxalate by high-performance liquid chromatography. Glassy carbon electrodes that have been cleaned by polishing should be electrochemically pre-treated using a satisfactory procedure such as the one described here before they are used for making analytical measurements. If this is not carried out then conditioning occurs naturally, inefficiently and indeterminately as the electrode is being used. This results in a steady increase in signal size, which is most marked immediately after the electrode has been polished and a fresh carbon surface has been produced. The occurrence of this gradual conditioning process in an HPLC system whilst eluent is flowing would result in a change of base line, as the base line also increases on conditioning the electrode, and this might be taken by chromatographers as a "settling down" period for the detector system.

It seems likely that electrochemical pre-treatment of glassy carbon electrodes will be shown to affect markedly the redox signals obtained with many other determinands. The general precision and reliability of amperometric detectors should be improved as a result of adequate electrochemical pre-

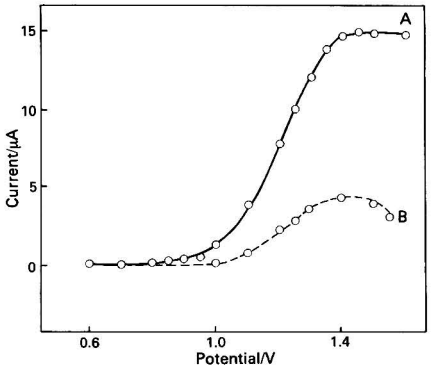


**Fig. 1.** Hydrodynamic voltammogram obtained at a glassy carbon electrode, A, that had been polished and then electrochemically pre-treated using the recommended procedure and B, that had been newly polished but not pre-treated. Oxalic acid dihydrate concentration = 63 μg $ml^{-1}$; flow rate = 6.0 ml $min^{-1}$

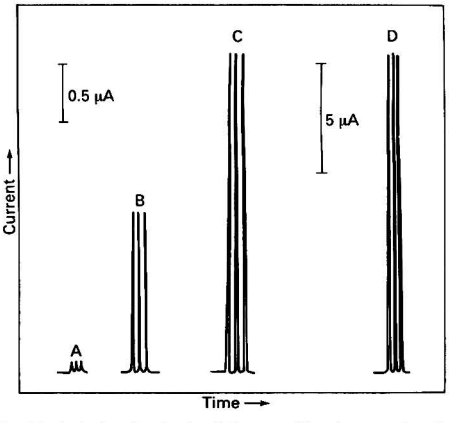


**Fig. 2.** Typical signals obtained for a calibration graph using a pre-treated electrode. Oxalic acid dihydrate concentration: A, 0; B, 6.3; C, 12.6; and D, 63 μg $ml^{-1}$. Measurement potential = 1.5 V

treatment of the glassy carbon electrode. It is even possible that the catalytic effect of the pre-treated surface will allow other compounds that are not currently considered to be amenable to amperometric detection to be oxidised or reduced more readily at the electrode and to be rendered amenable to determination by this means.

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## References

1. Blaedel, W. J., and Jenkins, R. A., *Anal. Chem.*, 1975, **47**, 1337.
2. Chan, H. K., and Fogg, A. G., *Anal. Chim. Acta*, 1979, **111**, 281.
3. Gonon, F. G., Fombarlet, C. M., Buda, M. J., and Pujol, J. F., *Anal. Chem.*, 1981, **53**, 1386.
4. Van Rooijen, H. W., and Poppe, H., *Anal. Chim. Acta*, 1981, **130**, 9.
5. Engstrom, R. C., *Anal. Chem.*, 1982, **54**, 2310.
6. Ravinchandran, K., and Baldwin, R. P., *Anal. Chem.*, 1983, **55**, 1782.
7. Engstrom, R. C., and Strasser, V. A., *Anal. Chem.*, 1984, **56**, 136.
8. Fogg, A. G., Fernández-Arciniega, M. A., and Alonso, R. M., *Analyst*, 1985, **110**, 851.
9. Fogg, A. G., Fernández-Arciniega, M. A., and Alonso, R. M., *Analyst*, 1985, **110**, 1201.
10. Mayer, W. J., McCarthy, J. P., and Greenberg, M. S., *J. Chromatogr. Sci.*, 1979, **17**, 656.
11. Fogg, A. G., and Summan, A. M., *Analyst*, 1984, **109**, 1029.

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# 2′-Mercapto-4-propylacetanilide: an Alternative to Thionalide for Precipitating Lead from Weak Acid Solution

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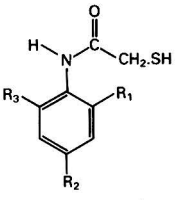
Six compounds containing the thioglycolic acid group (–NHCOCH$_2$SH) were compared with thionalide for precipitating microgram amounts of lead from acetate-buffered solutions at pH 5. Consistently higher recoveries (*ca.* 80%) were obtained with 2′-mercapto-4-propylacetanilide than with thionalide (*ca.* 60%).

**Keywords:** *2′-Mercapto-4-propylacetanilide; thionalide; mercaptoacetanilides; lead precipitation; trace elements*

Thionalide [2′-mercapto-*N*-2-naphthylacetamide] has been used for many years to determine those elements which can be precipitated, less conveniently, by hydrogen sulphide.[1] The procedure developed by Scott and Mitchell,[2–5] which uses it in combination with 8-hydroxyquinoline and tannic acid (penta-digalloylglucose), illustrates its application to trace element analysis. Thionalide is now difficult to obtain from commercial sources, however, because of the carcinogenic activity of the starting material, β-naphthylamine, and we have consequently tested a number of possible substitutes for it in Scott and Mitchell's procedure. The most effective so far identified is sodium sulphide, and a detailed comparison of that reagent with thionalide for the precipitation of lead and tin has been reported.[6] Concurrently with that investigation, several compounds containing the thioglycolic acid group (–NHCOCH$_2$SH) were synthesised and tested, in a search for a reagent having the same convenience of use as thionalide. One of these, 2′-mercapto-4-propylacetanilide (**I**), was found to be more effective than thionalide for precipitating lead from acetate-buffered solution at pH 5.2. The single-ring structure



I $R_1 = R_3 = H$; $R_2 = n\text{-}C_3H_7$
II $R_1 = R_3 = H$; $R_2 = O\text{—}n\text{-}C_3H_7$
III $R_1 = R_3 = CH_3$; $R_2 = H$
IV $R_1 = R_3 = H$; $R_2 = O\text{—}CH_2\text{—}Ph$
V $R_1 = R_3 = H$; $R_2 = O\text{—}\overset{O}{\overset{\|}{C}}\text{—}CH_2.SH$
VI $R_1 = R_2 = R_3 = H$

of 2′-mercaptoacetanilide (**VI**) was preferred as a basis for the reagents, on health grounds, to the double-ring structure of thionalide. Although a number of *N*-aryl mercaptoacetamides have been described,[7–10] there appear to be no previous reports of the analytical use (or of the preparation) of **I**. The main purpose of this paper is to draw attention to it because of its potential value for trace element analysis.

## Experimental

### Preparation of the Alternative Reagents

Avoiding the 2-naphthylamine unit contained in thionalide, a number of reagents were synthesised by a standard method[10]: 2′-mercapto-4-propylacetanilide (**I**), 2′-mercapto-4-propoxyacetanilide (**II**), 2,6-dimethyl-2′-mercaptoacetanilide (**III**), 2′-mercapto-4-benzyloxyacetanilide (**IV**), 4-aminophenol *N,O*-bis-2′-mercaptoacetate (**V**) and 2′-mercaptoacetanilide (**VI**). Their common feature was the basic structural unit **VI**, containing the thioglycolic acid group. Different aryl substituents were used in an attempt to find a lead complex that was relatively insoluble at pH 5.2. New compounds gave satisfactory analytical and infrared spectroscopic data consistent with their assumed structures.

### Precipitation Procedures

The precipitation procedures, using 8-hydroxyquinoline plus tannic acid and either thionalide or sodium sulphide, were detailed previously.[6] They utilise 8-hydroxyquinoline as the principal reagent to coprecipitate trace elements from solutions buffered to pH 5.2 with acetic acid and ammonium acetate, using aluminium as the collector. Tannic acid is needed to precipitate chromium, and thionalide (or sodium sulphide) to precipitate lead. In this work, the reagents substituted for thionalide were added in the same way, *i.e.*, as 2 ml of a 1% *m/V* solution in glacial acetic acid.

### Lead Determination

The precipitates were dried at 80 °C, ignited overnight at 450 °C and then analysed by atomic emission spectrometry using a cathode-layer carbon arc.[6] All the materials arced had a common matrix of hydrated alumina. However, in view of the earlier finding that calibration was affected by the specific combination of reagents used for precipitation, all the lead values in this work were determined from a calibration based

**Table 1.** Recovery of lead (15–150 μg) by coprecipitation with aluminium from acetate-buffered solutions at pH 5.2, using various reagent combinations as detailed in the text

| Precipitant | No. of precipitations | Mean recovery, % | Standard error, % |
|---|---|---|---|
| 8-Hydroxyquinoline (H) | 3 | 5 | 0.5 |
| H + tannic acid (HT) | 7 | 31 | 2.6 |
| HT + sodium sulphide | 22 | 101 | 1.8 |
| HT + reagent **I** | 17 | 75 | 1.6 |
| HT + thionalide | 11 | 55 | 2.1 |
| HT + reagent **II** | 6 | 39 | 0.8 |
| HT + reagent **III** | 2 | 28 | 1.1 |
| HT + reagent **IV** | 3 | 30 | 1.5 |
| HT + reagent **V** | 3 | 27 | 1.0 |
| HT + reagent **VI** | 4 | 27 | 0.9 |

**Table 2.** Distribution of recovered lead (45–150 μg) between precipitate and filtrate for several reagent combinations (see text)

| Precipitant | No. of precipitations | Precipitate: Mean recovery, % | Precipitate: Standard error, % | Filtrate: Mean recovery, % | Filtrate: Standard error, % |
|---|---|---|---|---|---|
| 8-Hydroxyquinoline (H) . . | 1 | 6 | 0.5 | 92 | 3.5 |
| H + tannic acid (HT) . . . . | 4 | 37 | 2.6 | 70 | 1.8 |
| HT + sodium sulphide . . . . | 3 | 97 | 2.4 | 1 | 0.2 |
| HT + reagent I . . . . . | 3 | 95 | 2.5 | 12 | 0.3 |
| HT + thionalide . . . . . | 6 | 62 | 1.7 | 38 | 0.8 |
| HT + reagent II . . . . . | 3 | 39 | 1.1 | 68 | 2.3 |

on standards precipitated with 8-hydroxyquinoline plus tannic acid and sodium sulphide.

## Results

The recovery of lead, added in amounts ranging from 15 to 150 μg, is shown in Table 1 for precipitations with ten different combinations of reagents. Only a few experiments were made with the ineffective reagents (**III**–**VI**). These four reagents gave recoveries that were comparable to those that were obtained with 8-hydroxyquinoline plus tannic acid, which were variable (*cf.*, Table 2). Although reagent **I** was more effective than thionalide, it did not give a full recovery.

A few experiments were carried out in which filtrates were also analysed by a further precipitation with 8-hydroxyquinoline plus tannic acid and sodium sulphide.[6] The distribution of recovered lead between the filtrate and the initial precipitate, obtained with the different reagents, is shown in Table 2. The lead recoveries in the filtrates are not affected by calibration bias,[6] and they confirmed not only that reagent **I** was better than thionalide, but also that neither of these reagents was as good as sodium sulphide.

## Discussion

When sodium sulphide is substituted for thionalide in the procedure described by Mitchell and Scott[4] for coprecipitating trace elements with aluminium, some operations must be carried out in a fume-cupboard, which can be inconvenient. One objective of this study, therefore, was to find a reagent that was at least as effective as thionalide for precipitating lead under the general conditions of their procedure. Apart from the ability to precipitate lead from acetate-buffered solutions at pH 5.2, additional requirements for any suitable substitute for thionalide were (*i*) that it should be easy to prepare and purify in 50-g amounts, using readily available materials, and (*ii*) that it should be stable at room temperature and convenient to use in the open laboratory. The poor recoveries of lead found with compounds **II**, **III**, **V** and **VI** were assumed to be due to the solubility of their lead complexes in the reaction medium. Compound **IV** was found to be poorly soluble in acetic acid and was discounted as a practical reagent for that reason. It is interesting that the replacement of the 4-propyl substituent in **I** by a 4-propoxy group in **II** gave a lower recovery of lead, as it was expected that the more electronegative substituent would have enhanced the stability of the lead complex.

It is concluded that 2′-mercapto-4-propylacetanilide (**I**), which has no objectionable odour, could be substituted for thionalide in Scott and Mitchell's procedure if the disadvantages of working with sodium sulphide outweigh the need for the complete recovery of lead that can be obtained with that reagent. The usefulness of reagent **I** as a more general substitute for thionalide in trace-element analysis merits a fuller investigation, but this is not planned by us in the foreseeable future.

## References

1. Welcher, F. J., "Organic Analytical Reagents," Volume IV, Van Nostrand, New York, 1948.
2. Scott, R. O., and Mitchell, R. L., *J. Soc. Chem. Ind.*, 1943, **62**, 4.
3. Mitchell, R. L., and Scott, R. O., *J. Soc. Chem. Ind.*, 1947, **66**, 330.
4. Mitchell, R. L., and Scott, R. O., *Spectrochim. Acta*, 1948, **3**, 367.
5. Mitchell, R. L., *Mikrochem. Mikrochim. Acta*, 1951, **36/37**, 1042.
6. Burridge, J. C., and Hewitt, I. J., *Analyst*, 1985, **110**, 795.
7. Weiss, U., *J. Am. Chem. Soc.*, 1947, **69**, 2682.
8. Bhandari, C. S., Mehnot, U. S., and Sogani, N. C., *J. Prakt. Chem.*, 1971, **313**, 849.
9. Bhandari, C. S., and Sogani, N. C., *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, 1972, **20**, 377.
10. Bateja, S., Verma, S., Bhandari, C. S., and Sogani, N. C., *J. Prakt. Chem.*, 1979, **321**, 134.

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# Titrimetric Determination of Catecholamines and Related Compounds Via Bromine Oxidation and Substitution

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A titrimetric method for the determination of 0.1–10 mg of catecholamines and related compounds as pure substances and in their dosage forms was investigated and found to offer improvements with regard to ease, speed and accuracy. The method is based on bromine oxidation of the catecholamines (adrenaline, noradrenaline, L-dopa, dopamine and methyldopa) to the corresponding benzoquinones; related compounds (octopamine, tyramine and tyrosine) undergo substitution to the dibromoaryl hypobromites. The benzoquinones and hypobromites oxidise iodide to liberate stoicheiometric amounts of iodine, which can be determined titrimetrically with thiosulphate, using starch as an indicator.

**Keywords:** *Catecholamine determination; bromine oxidation; bromination; titrimetry*

We have recently reported the bromine oxidation of some organic compounds containing two hydroxy groups in *ortho* or *para* positions to each other,[1] and bromine substitution of hydroxy compounds through the formation of hypobromites.[2] We have now extended these techniques to the determination of catecholamines and related compounds.

Increasing efforts are being directed towards the development of simple and reliable analytical techniques for the determination of medically and biologically important catecholamines and related compounds. Some titrimetric methods have been reported for the determination of adrenaline and noradrenaline,[3–6] L-dopa,[6–8] dopamine and methyldopa.[6] However, to our knowledge, the literature contains no suitable titrimetric procedure for the determination of octopamine, tyramine or tyrosine.

The purposes of the present investigation were to develop a simple assay for catecholamines and related compounds using bromine water and to apply the procedure to various dosage forms. The method is based on the oxidation of catecholamines (adrenaline, noradrenaline, dopa, dopamine and methyldopa) with an excess of bromine water to the corresponding benzoquinones; octopamine, tyramine and tyrosine undergo substitution to form the dibromoaryl hypobromites. On treatment of the benzoquinones or hypobromites with iodide, equivalent amounts of iodine are liberated and determined titrimetrically with thiosulphate. The excess of bromine is removed with formic acid.

## Experimental

### Reagents

All chemicals used were of analytical-reagent grade.

*Catecholamines and related compounds.* Sample solutions containing 2 mg $ml^{-1}$ of each compound were prepared and diluted with distilled water as required. All compounds (Fluka) had a stated purity of not less than 99% of the active ingredient present, except noradrenaline and methyldopa, which were not less than 98% pure. The tablets were weighed and powdered, dissolved in about 20 ml of 0.1 N hydrochloric acid, filtered into a calibrated flask and diluted to volume with distilled water.

*Sodium thiosulphate solutions, 0.01 and 0.001 N.* These were prepared and standardised against potassium iodate solutions of similar concentration.

*Other solutions.* Solutions of bromine water (saturated), formic acid (concentrated) and starch (1%) were used.

### Procedure

Into a 100-ml Erlenmeyer flask, introduce an accurately measured volume of sample solution containing 0.1–10 mg of the sample compound in the pure form or as the dosage form. Dilute with water to make a total volume of about 15 ml. Add 3 ml of bromine water, stopper the flask and shake it for 1 min. Remove the stopper and destroy the excess of bromine with 2 ml of formic acid (the solution becomes colourless). Add about 0.3 g of potassium iodide and titrate the liberated iodine with 0.01 N sodium thiosulphate solution in the usual way, using starch as an indicator. For low concentrations (less than 2 mg of determinant) use 0.001 N sodium thiosulphate solution. Run a blank determination under identical conditions but with no active ingredient present.

With this procedure, 1 ml of 0.01 N sodium thiosulphate solution is equivalent to 0.9161 mg of adrenaline, 0.8459 mg of noradrenaline, 0.9860 mg of dopa, 0.9482 mg of dopamine hydrochloride, 1.1462 mg of methyldopa, 0.9482 mg of octopamine hydrochloride, 0.9060 mg of tyrosine and 0.8682 mg of tyramine hydrochloride.

## Results and Discussion

Catecholamines are readily and quantitatively oxidised by bromine water to the corresponding benzoquinones. However, the related compounds (containing one hydroxy group) undergo substitution to the corresponding dibromoaryl hypobromites.

### Effect of Amount of Bromine

The results showed that 3 ml of saturated bromine water were sufficient for the rapid and quantitative reaction of up to 10 mg of the catecholamine or related compound. Greater excesses of bromine increased the blank value. Complete removal of the excess of bromine was achieved with 2 ml of formic acid.

### Reaction Time

Oxidation of catecholamines or substitution of the related compounds by bromine water proceeds to completion within 1 min; a reaction time of up to 20 min had no significant effect on the results.

### Effect of Volume of Water

The method was applied to the determination of 0.1–10 mg of the compounds of interest in a total volume of about 15 ml.

However, low results were obtained for amounts less than 0.5 mg of the studied compounds in this volume, which may be attributed to the incomplete reaction in dilute solutions.

### Liberation Time of Iodine

It was found that on addition of iodide to the benzoquinone or hypobromite formed, iodine was liberated rapidly and quantitatively within 1 min; a standing time of up to 10 min had no effect on the results. The end-point, using starch as an indicator, was stable for about 3 min. It should be noted that the average blank value is 0.03 ml of 0.01 N sodium thiosulphate solution.

**Table 1.** Accuracy and precision of the method

| Compound | Amount taken/mg | Mean recovery,* % | Coefficient of variation,* % |
|---|---|---|---|
| Adrenaline | 0.1 | 97.9 | 1.5 |
| | 1.0 | 99.2 | 0.6 |
| | 10.0 | 98.3 | 0.3 |
| Noradrenaline | 0.1 | 98.2 | 1.3 |
| | 1.0 | 99.9 | 0.0 |
| | 10.0 | 100.2 | 0.1 |
| L-Dopa | 0.1 | 98.0 | 1.7 |
| | 1.0 | 100.1 | 0.2 |
| | 10.0 | 100.2 | 0.4 |
| Dopamine hydrochloride | 0.1 | 98.7 | 2.1 |
| | 1.0 | 99.8 | 0.5 |
| | 10.0 | 99.2 | 0.3 |
| Methyldopa | 0.1 | 100.3 | 1.8 |
| | 1.0 | 100.0 | 0.0 |
| | 10.0 | 100.3 | 0.2 |
| Octopamine hydrochloride | 0.1 | 98.6 | 2.3 |
| | 1.0 | 99.8 | 0.7 |
| | 10.0 | 99.0 | 0.4 |
| Tyramine hydrochloride | 0.1 | 98.5 | 0.6 |
| | 1.0 | 100.0 | 0.0 |
| | 10.0 | 100.1 | 0.0 |
| Tyrosine | 0.1 | 98.5 | 0.6 |
| | 1.0 | 99.9 | 0.1 |
| | 10.0 | 98.9 | 0.1 |

* Five determinations.

**Table 2.** Determination of catecholamines in some pharmaceutical preparations

| Sample | Catecholamine | Amount: Stated by the manufacturer | Amount: Found by the present method |
|---|---|---|---|
| Adrenaline* | Adrenaline | 1 mg ml$^{-1}$ | 1 ± 0.05 mg ml$^{-1}$ |
| Epifrin† | Adrenaline | 2 % | 2 ± 0.2 % |
| Arterenol‡ | Noradrenaline | 1 mg ml$^{-1}$ | 1 ± 0.05 mg ml$^{-1}$ |
| Aldomet§ | Methyldopa | 250 mg | 250 ± 0.7 mg |

* Adrenaline ampoules, labelled to contain 1 mg ml$^{-1}$ of adrenaline. Gedeon Richter, Budapest, Hungary.

† Epifrin ophthalmic solution, labelled to contain 2.0% of adrenaline. Allergan Pharmaceuticals, Irvine, CA, USA.

‡ Arterenol ampoules, labelled to contain 1 mg ml$^{-1}$ of noradrenaline. Hoechst Laboratories, Frankfurt, FRG.

§ Aldomet tablets, labelled to contain 250 mg of methyldopa per tablet. Merck, Sharp and Dohme, Rahway, NJ, USA.

### Interferences

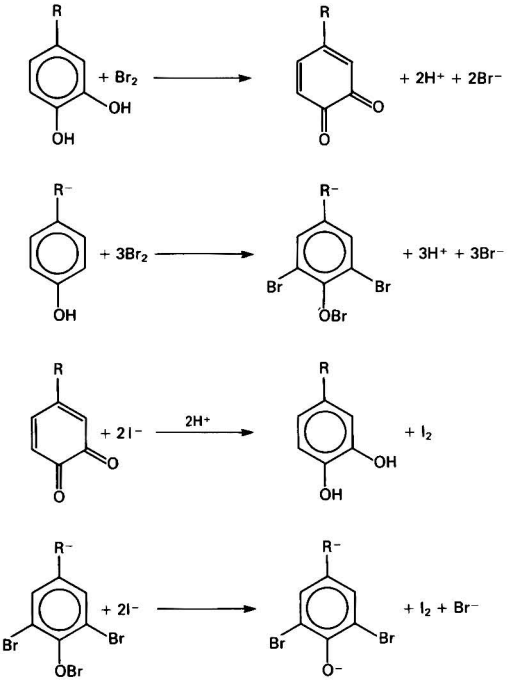
All compounds that undergo bromine oxidation or substitution will interfere, *e.g.*, amines, aminophenols, catechol, hydroquinone, resorcinol and cresols.

### Accuracy and Precision

Under the optimised conditions mentioned, the accuracy and precision of the method were checked. The results (five replicate determinations) are given in Table 1, and indicate that the method is reliable. Table 2 shows application of the method to some pharmaceutical preparations containing catecholamines. We always used at least five tablets for each sample preparation.

### Proposed Reactions

When a compound contains two hydroxy groups in *ortho* positions (as in catecholamines), it will not combine with bromine but undergoes oxidation to the corresponding benzoquinone,[1] which oxidises equivalent amounts of iodide to iodine. However, phenols (octopamine, tyramine and tyrosine) undergo bromination in the unoccupied *ortho* and *para* positions to form the dibromo compound, and with an excess of bromine the substitution proceeds further to form the corresponding dibromoaryl hypobromites, which react with iodide to liberate equivalent amounts of iodine.




R = —CH(OH)$CH_2NHCH_3$ (adrenaline),—CH(OH)$CH_2NH_2$ (noradrenaline),—$CH_2CH(NH_2)COOH$ (dopa),—$CH_2CH_2NH_2$ (dopamine)or

$CH_2C$(CH$_3$)(NH$_2$)—COCH (methyldopa). R′ = $CH_2CH_2NH_2$ (tyramine),

—$CH_2CH(NH_2)COOH$ (tyrosine) or—CH(OH)$CH_2NH_2$ (octopamine)

## References


1. Amin, D., *Analyst,* 1985, **110**, 211.
2. Amin, D., and Bashir, W. A., *Talanta,* 1984, **31**, 283.
3. Abou Ouf, A., Walash, M. I., and Salem, F. B., *Analyst,* 1981, **106**, 949.
4. "United States Pharmacopeia, Nineteenth Revision," Mack, Easton, PA, 1975, pp. 274 and 320.
5. Subert, J., Bachrata, M., Knazko, L., and Stemborova, A., *Cesk. Farm.,* 1975, **24**, 68.
6. Greenhow, E. J., and Spencer, L. E., *Analyst,* 1973, **98**, 485.
7. "British Pharmacopeia 1973," HM Stationery Office, London, 1973, pp. 263 and 264.
8. Jouin, J., and Sains, E., *Ann. Pharm. Fr.,* 1972, **30**, 139.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# COMMUNICATION

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

*Manuscripts are usually examined by one referee and inclusion of a Communication is at the Editor's discretion.*

## Supported Chemoreceptive Lipid Membrane Transduction by Fluorescence Modulation: the Basis of an Intrinsic Fibre-optic Biosensor

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**Keywords:** *Lipid membrane; biosensor; chemoreceptor; fluorescence; evanescent wave*

Dedicated selective chemical analysis has become one of the forefronts of analytical research, and numerous new technologies and devices have been reported.[1,2] This group previously introduced the manipulation of the properties of ordered bilayer lipid membranes (BLM) for implementation of a transduction mechanism suitable for electrochemical sensing of membrane-embedded selective receptor binding events.[3-5] Organised lipid structures have tremendous analytical potential as transducers owing to the sensitivity of the membrane structure to interactions with proteins, and as the membrane environment can often optimise the functional binding characteristics of embedded receptors. The recent identification of the lipid membrane parameters that can be readily perturbed by selective receptor complexation[4] makes possible their analytical exploitation in non-electrochemical modes.

One useful transduction strategy could employ fluorescence variations of membrane-associated fluorophores, which are sensitive to surface charge, dipolar potential and lipid mobility.[6] Practical limitations in the exploitation of this membrane technology originate with instability of the lipid membrane and the lack of convenient and reliable instrumentation for probing membrane structure. Previous work by McConnell and co-workers[7,8] and Andrade *et al.*[9] provides evidence that membranes can be stabilised on to surfaces suitable for direct optical analysis. This work demonstrates that phospholipid membranes can be interfaced to borosilicate glass surfaces in a stable and reproducible manner. The fluorophore 1-anilinonaphthalene-8-sulphonate (ANS) was shown to be sensitive to supported membrane structures, and reported membrane perturbations caused by the membrane probes phloretin and valinomycin. These results demonstrate that a fluorescence-based chemoreceptive transduction strategy is possible. A membrane-coated fibre-optic biosensor that maximises membrane stability and transduction sensitivity is described.

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## Experimental

### Reagents

Phosphatidylcholine from egg yolk (Avanti Biochemicals, Birmingham, AL, USA) and cholesterol (Sigma, St. Louis, MO, USA) were used for lipid membrane formation. The fluorescent agent 1-anilinonaphthalene-8-sulphonate (ANS) (Eastman Kodak, Rochester, NY, USA) and membrane probes phloretin and valinomycin (Sigma) were all used as received. All solvents were of analytical-reagent grade, and all water used was purified to remove trace organics and was doubly distilled.

### Apparatus

A Lauda Model 1974 thin-film balance (Brinkman, Toronto, Canada) was used in association with an in-house film lift[10] for deposition of lipid monolayers on to glass wafer surfaces. Glass wafers were cut to dimensions of 0.5 × 3 cm from plain borosilicate glass microscope slides of thickness 1.0 mm (J. B. EM Services, Dorval, Canada).

Surface analysis was accomplished with an Auto EL II ellipsometer (Rudolph Research, Flanders, NJ, USA) and an electron spectrometer, using an Mg Kα source. Contact angle estimates were obtained from an in-house assembly consisting of a horizontally mounted low-power optical microscope with variable stage height, background illuminator and photographic attachment. All measurements were derived from photographic records.

Fluorescence studies were made with a Model 204-A spectrofluorimeter (Perkin-Elmer, Norwalk, CT, USA), where the sample wafers were inserted into standard quartz cuvettes.

### Procedures

The glass wafers were scored with a diamond knife and then cleaved to the required size. These wafers were washed with sodium dodecysulphate detergent, treated with chromic acid

for periods of up to 1 h and then rinsed extensively with copious amounts of water. Equal mass ratios of phospholipid and cholesterol were used in deposition experiments. These surfactants were prepared as solutions containing approximately 4 mg of total lipid in 5 ml of hexane. Approximately 70 μl of this solution were deposited slowly by syringe on to the aqueous sub-phase in the Lauda trough. After a period of at least 10 min, the monolayer was compressed to a pre-selected value, which was then maintained by operation in an automatic constant film pressure mode. The sub-phase was varied between experiments, and was chosen to contain distilled water or 0.1 M KCl solution or an aqueous solution of $10^{-4}$ M ANS. Monolayer transfer to a wafer was accomplished by immersion and withdrawal of the substrate through the air - water interface at linear casting rates of approximately 0.5 cm $min^{-1}$. The wafers were investigated for fluorescence by mounting the latter in conventional quartz cuvettes filled with water. Variability was observed in the fluorescence signal as a function of the orientation angle of the wafers to the source, and experimental results are reported for a constant angle of 45°. Phloretin and valinomycin membrane probes were prepared as concentrated methanolic solutions, and were then added to sample cuvettes at concentrations of $10^{-5}$ and $10^{-6}$ M, respectively. Investigation included the use of blank samples where only methanol was added to cuvettes containing coated wafers.

## Results and Discussion

### Lipid Membrane Deposition

Of fundamental importance to the proposed sensing strategy is the character of the organised lipid membrane. The primary advantage of this matrix rests in its ability to provide an interior environment for fluorescent agents that is sensitive to surface perturbation, so that membrane surface interactions with analytes of interest can potentially be transduced into fluorescence variations.[6]

Deposition of conventional BLM-forming phospholipid - steroid mixtures was attempted on prepared glass wafers. Deposition involved transfer of lipid monolayers from an air - water interface to the substrate by substrate penetration through the organised lipid assemblies while maintaining constant membrane surface pressure to ensure membrane structural reproducibility. Transferred membranes remained on substrate surfaces by means of weak physical and chemisorptive processes rather than by covalent linkage. The character of deposited membranes was investigated to establish criteria for the evaluation of film quality, *i.e.*, surface hydrophobicity, thickness, homogeneity of coverage and lipid packing density.

The use of simple visual probes such as a fine dispersion of the mineral talc dusted on to the surface of a compressed floating monolayer readily demonstrated whether monolayer transfer to a substrate actually occurred. Visual results indicated that the maximum surface area of phospholipid monolayer transferred on to the substrates occurred on the first withdrawal from the sub-phase. Ellipsometric investigation determined that subsequent complete cycles of deposition did not result in multilayer formation. Contact angle approximations were obtained and clearly showed that the substrates employed in this work could physically retain a monolayer of lipid with head-groups interfacing to the glass and hydrophobic chains oriented away from the interface. Contact angle estimates range from 10 ± 3° for cleaned glass surfaces, to 55 ± 5° for glass wafers coated with lipid at monolayer pressures maintained at 35 mN $m^{-1}$. Comparison of coated glass wafer contact angle with monolayer surface pressure in the trough also produced a correlation ranging from approximately 40 to 55° over compression pressures of 20–35 mN $m^{-1}$. The compression curve for these phospholipid membranes has been carefully studied and showed no discontinuities or inflections before collapse pressures of 40 mN $m^{-1}$ were attained.[5] This implies that the structure of the monolayer on the trough was directly reflected in the character of the supported film, and provides a means of engineering a deposited monolayer structure. Preliminary results of the time dependence of the deposited phospholipid monolayer structure as estimated from contact angle measurements indicated no significant alteration over a period of 24 h. The formation of bilayers or multilayers is more difficult to achieve. The presence of a strong electrolyte sub-phase such as 0.1 M KCl or $10^{-4}$ M concentrations of divalent species such as $Ba^{2+}$ and $Ca^{2+}$, useful for repetitive stearic acid deposition,[11] does not significantly influence phospholipid multilayer formation.

### Supported Lipid Membrane Fluorescence

A common hydrophobic fluorescent probe of membrane interior hydrophobicity and order is ANS.[12] This probe is believed to reside within the non-polar region of a lipid membrane, although its exact location has not been conclusively proved.[6] The quantum yield of this particular fluorophore is sensitive to molecular mobility within membranes, where increased mobility causes greater loss of excitation energy through non-radiative collisionally based processes. This probe has been previously reported in vesicular phospholipid suspensions in aqueous solutions,[12] and here we extended the investigation of its properties to supported phospholipid systems.

The fluorescent emission of ANS, which can be intense even in dilute aqueous solution, is centred at approximately 510 nm, and excitation is optimised by sample irradiation at 365 nm. Clean glass wafers placed in cuvettes containing water and irradiated at the latter excitation wavelength in the spectrofluorimeter indicated no significant emission signal in the 100 nm range centred on 510 nm. Similar results were obtained for monolayer-coated glass wafers. Spectroscopic investigation of glass wafers dipped into an aqueous solution of $10^{-4}$ M ANS indicated that fluorescence was present at very low intensity, leading to the conclusion that ANS does not selectively bind to the hydrophilic glass surface. Glass wafer deposition of a lipid monolayer floated over a similar ANS solution produced supported lipid membranes that could generate measurable fluorescence intensities when placed in cuvettes containing water, even though only monolayer coverage was present. The small fluorescence signal profile necessitated measurement by integration above an established base line. Widely variable fluorescence intensity could be measured for deposition on to different glass wafers at a fixed high monolayer compression pressure of approximately 35 mN $m^{-1}$. Analysis of the relative fluorescence values clearly indicated a bimodal distribution where approximately 25% of coatings produced fluorescence comparable to blank reference standards, while the other 75% demonstrated consistent and reproducible fluorescence signals. This indicated that even though monolayers could be transferred to glass wafers by casting from a trough, they could subsequently be floated from the glass when the wafers were immersed in the water in the sampling cuvettes. The physical feature responsible for adhesion or lack of adherence to the glass surface has not yet been identified. It was possible to observe fluorescence from monolayer-coated wafers that were not placed in water, but the emphasis of this work was directed towards solution-based interfacing of supported lipid membranes for sampling purposes.

Table 1 represents fluorescence observed from monolayers maintained at significantly different compression pressures during transfer to the glass wafer. These results clearly demonstrate that fluorescence was related to the supported membrane structural characteristics, and that these characteristics could be defined on a trough and maintained after monolayer transfer to the support. Time studies indicated that

**Table 1.** Fluorescence measurements for lipid monolayers supported on glass

| Sample description | Relative fluorescence signal |
|---|---|
| Clean glass wafer | 0–1 |
| Clean wafer after immersion in $10^{-4}$ M ANS | $2 \pm 1$ |
| Monolayer coated wafer cast in $10^{-4}$ M ANS sub-phase for compression pressure: | |
| 20 mN $m^{-1}$ | $1 \pm 1$ |
| 25 mN $m^{-1}$ | $2 \pm 1$ |
| 30 mN $m^{-1}$ | $4 \pm 1$ |
| 35 mN $m^{-1}$ | $14 \pm 3$ |

**Table 2.** Fluorescence response of supported lipid monolayers to phloretin and valinomycin

| Membrane perturbant | Relative fluorescence signal | | |
|---|---|---|---|
| | Initial | After 5 min | After 1 h |
| Phloretin ($10^{-5}$ M) | $14 \pm 3$ | $7 \pm 2$ | $5 \pm 2$ |
| Valinomycin ($10^{-8}$ M) | $10 \pm 1$ | $2 \pm 1$ | $2 \pm 1$ |

only a slight decrease in fluorescence signal occurred after periods up to 24 h, and this was most prevalent in membranes cast at high compression pressures. Even though this may be related to loss of structural order in such membranes, further experimentation is required to determine if ANS is slowly extracted from the supported membrane into the aqueous cuvette environment, resulting in a fluorescence decrease. Spectroscopic investigation of the water in the sample cuvettes after fluorescence analysis of wafers showed no ANS fluorescence; however, the fluorophore must have been in very low concentration in a medium unsuitable for an efficient quantum yield.

We have proposed that receptors act primarily on ordered lipid structures by local dipolar potential and packing/fluidity perturbation.[5] In order to probe fluorescence alteration of supported lipid monolayers as a function of these latter parameters, the experimentation included membrane interaction with the dipolar potential probe phloretin and the structural perturbant valinomycin. Phloretin (relative molecular mass 275) is known to partition into the lipid membrane polar zone, and has a large dipole moment ($\mu = 5.6$ D) known to align against the inherent transmembrane potential.[13] Valinomycin (relative molecular mass 1111) is a cyclic polypeptide capable of complexing Group I cations and dissolving into the hydrocarbon phase of lipid membranes where it causes structural disorder.[14] These two agents at concentrations of $10^{-5}$ M and $10^{-8}$ M, respectively, provided no fluorescence signal in the required analytical range when they were added as methanolic solutions to the water of a sample cuvette. Table 2 summarises the substantial effect that these membrane probes had on the fluorescence of supported lipid membranes coated at 35 mN $m^{-1}$ presure. The effect of both probes was to cause a net decrease in fluorescence, implying they caused substantial alteration of membrane structure and/or electrostatics.

### Fluorescent Membrane-based Chemical Sensor

The chemistry of the sensing strategy is the crucial factor determining the selectivity and sensitivity of the technique, although instrumental configuration will also impose physical measurement limits. This work has demonstrated that lipid membranes can reproducibly be supported and stabilised on conventional substrates for the purpose of chemical transduction by fluorescence. One problem faced by the described fluorescence strategy is the difficulty in achieving a suitable device configuration that would eliminate complications of optical system interfacing to the proposed chemically selective surface. We are currently investigating an intrinsic method based on fibre-optic technology. The chemical - instrumental interface consists of a receptor and fluorophore-modified lipid membrane, coated directly on to the surface of an optical fibre. Monochromatic light chosen to stimulate the fluorophore is transmitted by total internal reflection through the optical fibre. The evanescent wave experienced at each reflection acts as the optical stimulant penetrating completely through the membrane coating. The resulting fluorescence, controlled by selective receptor binding of stimulant, is transmitted by the fibre to a detector. Appropriate fluorophores can be embedded in the lipid matrix or conjugated to the selective receptor. This provides a practical sensor device strategy optimising the use of stabilised lipid membrane technology.

We are indebted to the Department of Chemistry, University of Toronto, for providing a fellowship to C. B., and to Allied Canada Inc. for providing access to the equipment used for monolayer casting and characterisation.

## References

1. Thompson, M., and Krull, U. J., *Trends Anal. Chem.*, 1984, **3**, 173.
2. Seiyama, T., Fueki, K., Shiokawa, J., and Suzuki, S., *Editors*, "Proceedings of International Meeting on Chemical Sensors, Fukuoka, Japan," Elsevier-Kodansha, New York, Tokyo, 1983.
3. Thompson, M., and Krull, U. J., *Anal. Chim. Acta*, 1983, **147**, 1.
4. Krull, U. J., and Thompson, M., *Trends Anal. Chem.*, 1985, **4**, 90.
5. Krull, U. J., Thompson, M., Vandenberg, E. T., and Wong, H. E., *Anal. Chim. Acta*, 1985, **174**, 83.
6. Yguerabide, J., and Foster, M. C., *in* Grell, E., *Editor*, "Molecular Biology, Biochemistry and Biophysics," Vol. 31, Springer-Verlag, New York, 1981, p. 200.
7. Weis, R. M., Balakrishnan, K., Smith, B. A., and McConnell, H. M., *J. Biol. Chem.*, 1982, **257**, 6440.
8. Tamm, L . K., and McConnell, H. M., *Biophys. J.*, 1985, **47**, 105.
9. Andrade, J. D., Van Wagenen, R. A., Gregonis, D. E., Newby, K., and Lin, J.-N., *IEEE Trans. Electron Devices*, 1985, **32**, 1175.
10. Krull, U. J., Thompson, M., and Wong, H. E., *Analyst*, 1985, **110**, 1299.
11. Blodgett, K. B., and Langmuir, I., *Phys. Rev.*, 1937, **51**, 964.
12. Radda, G. K., and Vanderkooi, J., *Biochim. Biophys. Acta*, 1972, **265**, 509.
13. Reyes, J., Greco, F., Motais, R., and Latorre, R., *J. Membr. Biol.*, 1983, **72**, 93.
14. Thompson, M., and Krull, U. J., *Anal. Chim. Acta*, 1982, **141**, 33.

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# BOOK REVIEWS

**Gas und Flussigkeits-Adsorptionschromatographie**
A. V. Kiselev and Ja. I. Jašin. Pp. 391. VEB Deutscher Verlag der Wissenschaften. 1985. ISSN 0079 1997.

The present book is a German translation (published in East Germany) of a work that was issued originally in Moscow in 1979. It contains ten chapters—an Introduction to the subject, followed first by chapters on gas (including capillary) chromatography, and then by chapters covering liquid adsorption chromatography. There are sections dealing with apparatus, methods, selectivity for both techniques and a chapter about the theory and use of the Henry constant and Kováts retention index, and their relationship with different types of column packings.

The efficiency and applications of each of the techniques are covered. The book is described as being on the theory and practice of adsorption chromatography and it is fair to say that the theoretical aspect is covered particularly well. Practical details are given where necessary and are illustrated by good, clear chromatograms. The applications given are drawn from the fields of biochemistry, food, pharmaceuticals and related areas, and include a considerable number of helpful examples of the analysis of gases and solvents. Other types of compound to which reference is made include glycosides, phenols, vitamins, antibiotics and diazepam and its derivatives, with many others for which tables of references are provided.

The volume contains 184 illustrations and 35 tables. It has between 1500 and 2000 references, which are arranged conveniently by chapter at the end of the book, with a list provided at the start of the symbols used in the text and a reasonable index.

Essentially it is a review volume with theory rather than a practical handbook, but it should find a place on the library shelf of both academic and industrial research establishments and be a very useful addition to the reference libraries of German-speaking chromatographers.

*D. Simpson*

**Stripping Analysis. Principles, Instrumentation, and Applications**
Joseph Wang. Pp. viii + 160. VCH Publishers, Deerfield Beach. 1985. Price DM120. ISBN 0 89573 143 6 (VCH Publishers); 3 527 26192 3 (VCH Verlagsgesellschaft, Weinheim).

This is the first monograph on electrochemical stripping techniques to appear for well over a decade and the author has been very successful in his aim of summarising recent advances in the field. He reports extensively on the development of both commercial and laboratory-constructed instrumentation, with emphasis on rapid stripping techniques, such as square-wave voltammetry, fast linear scan voltammetry and potentiometric stripping analysis, and also instrumental computerisation. A comprehensive account is given of the increasing importance of flow systems as opposed to batch systems. The design of the various flow cells used in electrochemical stripping measurements, such as wall-jet cells, thin-layer cells and dual electrode cells, is described in detail and the advantages and drawbacks of the different designs are discussed briefly. Reduction of stripping potential overlap by medium exchange in flow systems is illustrated by means of several examples. Two of the most recent trends in stripping analysis, namely the determination of electroactive organic compounds or metal chelates by means of cathodic stripping voltammetry, subsequent to adsorptive accumulation, and the use of chemically modified solid electrodes, are also illustrated. In the final chapters a complete survey of all applications, prior to 1985, of stripping analysis is given, focusing on trace metal determinations in body fluids, foodstuffs and environmental samples.

The book can be highly recommended to those who are interested in recent developments in stripping analysis and to those considering the commencement of stripping analysis in their laboratories. Those who are interested in a detailed theoretical treatment of the theory of stripping analysis will need to look elsewhere.

*Daniel Jagner*

**Maximum Concentrations at the Workplace and Biological Tolerance Values for Working Materials 1984**
Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. Report No. XX. Pp. 80. Verlag Chemie. 1985. Price DM23. ISBN 3 527 27332 8 (Verlag Chemie); 0 89573 379 X (Verlag Chemie International).

Practitioners in the field of health and safety at work will be aware of the benefits of examining the policies and guidelines developed in other countries. This is especially true for air pollution, where too often the real situation can be compared with the idealised guidelines only with difficulty and a great deal of interpretive skill. As a practitioner in Britain who for official purposes must work with the Occupational Exposure Limits of the Health and Safety Executive, I wholeheartedly recommend this, the German equivalent, as a valuable adjunct and aid.

The essential list of compounds and their maximum concentrations are well presented, with good spacing, structural formulae and even line drawings for the more complex molecules. Where appropriate, compounds are flagged with symbols to indicate the dangers from sensitisation or cutaneous absorption. Also, the saturated vapour pressures for highly volatile compounds at 20 °C are quoted to indicate the potential hazard due to vaporisation from open vessels. Five categories of excursions above the time-weighted average concentrations are defined, and allocated as appropriate within the tables.

Nine pages are given to a concise description and discussion of mutagens and carcinogens and how to assess them. For substances that cannot be regarded as safe at any level of exposure, this section has a table of "Technical Guiding Concentrations," *i.e.*, concentrations at which the degree of risk may be regarded as acceptable. Special discussion is also devoted to the assessment and interpretation of hazards from dusts (7 pages), mixtures of compounds such as gasoline, turpentine and pyrolysis products, and metal working fluids. Consideration is given to the problem of dealing with compounds that promote a feeling of nausea without at the same time constituting a toxic hazard. One could wish for more guidance on this subject, but it is at least advantageous that it has been mentioned.

The section on "Biological Tolerance Values" has 6 pages. It deals with blood, plasma, urine and alveolar air, and includes advice on sampling technique.

In summary, this report is notable for its clear presentation, the concise and comprehensive treatment of its subject and its willingness to treat those difficult areas of assessment where the real situations cannot be fitted neatly into any idealised guidelines.

*B. I. Brookes*

**Pollutants and Their Ecotoxicological Significance**
Edited by H. W. Nürnberg. Pp. xiv + 515. Wiley. 1985. Price £49.50. ISBN 0 471 90509 7.

Increasingly there is concern about the proliferation of new chemical substances and their potential risks to humans and to the environment. One reaction to this concern is the development of legislation requiring new chemical products to be investigated thoroughly before manufacture, importation or marketing. The implications for the testing of these chemicals are considerable in view of the complex ecological chemistry involved. The 30 chapters in this book attempt to give a comprehensive review of the problems associated with this field of applied trace chemistry.

The book is divided into four sections, of which the first three deal with the atmosphere, the aquatic environment and the terrestrial environment. The final and smallest concentrates on regulatory aspects. Each chapter is written by experts in the relevant disciplines and the examples, based on their own experiences and expertise, are drawn from throughout Europe. The effect of this multi-national approach is to produce some sections where the translation into English makes hard reading.

With such a diversity of topics contained in one book, individual chapters could easily be criticised by specialists in each subject. Thus, for example, it is surprising that with the considerable interest in acid deposition, a review of sampling and analysis techniques for atmospheric acids occupies just over five pages of text, including the reference list.

The reviewer's criticisms are relatively minor, however, and there is no doubt that the book offers a well presented and valuable overview of an important interdisciplinary subject. Libraries serving environmental researchers will surely find a demand for this text, which it is pleasing to note has many of the lists of literature references citing recent work.

*R. S. Barratt*

# ERRATUM

## Automatic Two-stage Thermal Desorption Gas Chromatography for Low-volatility Organic Vapour Determination

**J. F. Alder, E. A. Hilderbrand and J. A. W. Sykes**
*Analyst*, 1985, **110**, 769–773

Page 773, Tables 5 and 6: the final sentence of the title should read "Cold trap and ATD 50 tubes packed with silanised glass-wool" in both instances.

corrected
ch. S.
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