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Accuracy of Determination of Cadmium, Copper, Lead, Nickel and Zinc in River Waters

Analytical Quality Control (Harmonised Monitoring) Committee*
Water Research Centre, P.O. Box 16, Henley Road, Medmenham, Marlow, Buckinghamshire SL7 2HD, UK

The Department of the Environment in collaboration with the Regional Water Authorities has initiated a Scheme for the Harmonised Monitoring of River Waters in England and Wales. The Scottish Development Department has been closely associated with the development of this scheme, and has introduced a similar scheme in Scotland in collaboration with the River Purification Boards. To achieve the required comparability of results, each laboratory takes part in an analytical quality control (AQC) scheme; this work is co-ordinated by the Water Research Centre. The general approach adopted to AQC has already been described, and this paper presents the results of tests made on the determination of cadmium, copper, lead, nickel and zinc in river waters. The accuracy requirements for these determinands [that the total error on a single result should be not larger than $10\ \mu g\ l^{-1}$ for Cu, Pb, Ni and Zn ($1\ \mu g\ l^{-1}$ for Cd) or 20% of the sample concentration, whichever is the greater], were not achieved by participating laboratories.

Keywords: *River water analysis; cadmium, copper, lead, nickel and zinc determination; accuracy of results; inter-laboratory comparability; analytical quality control*

The Scheme for the Harmonised Monitoring of the Quality of Inland Fresh Water has been described in detail.[1] It is intended to provide objective data on river water quality so that accurate assessments can be made of long-term trends in the qualities of rivers and of the amount of materials discharged by them to the sea. The Scheme complements monitoring carried out for regional or local purposes and one of its essential aims is to achieve comparability of the results from all participating laboratories. To that end, special investigations have been carried out to establish suitable sampling locations and to define the necessary sampling frequency. Sampling procedures have been recommended, and each participating laboratory carries a specially designed programme of tests to ensure that its analytical results are of adequate accuracy for the Scheme.

The Water Research Centre (WRC) is under contract to the Department of the Environment to advise on and to co-ordinate this analytical quality control (AQC) programme.

The need for, and details of an approach to, a planned AQC system for this and similar monitoring schemes have been discussed elsewhere.[2] In view of the growing interest in achieving comparable results from a number of laboratories, it was thought useful to describe the AQC work for the Harmonised Monitoring Scheme and to present the results for different determinands. This paper considers the determination of cadmium, copper, lead, nickel and zinc; earlier papers describe the work for chloride,[3] ammoniacal nitrogen,[4] total oxidised nitrogen and nitrite,[5] suspended solids[6] and pH and conductivity[7] and subsequent papers will deal with other determinands of importance in rivers.

Organisation of the Work

A Committee was formed to plan the collaborative work and has representatives† from the Department of the Environment, the Scottish Development Department, each Regional Water Authority (RWA), the Scottish River Purification Boards and WRC. This Committee decided to adopt the approach to AQC described elsewhere,[2] each determinand being studied in two phases.

Phase (i). One laboratory in each of the 10 RWAs and one in Scotland participated, the WRC acting as co-ordinating laboratory.[2]

Phase (ii). After satisfactory results have been obtained in Phase (*i*), those laboratories act as co-ordinators of tests within RWAs and in Scotland. Certain RWAs were not involved in this stage because all analyses for Harmonised Monitoring are made by one laboratory.

This report presents the results obtained in Phase (*i*).

Required Analytical Accuracy

The following requirements for analytical accuracy were agreed for the trace metals: maximum tolerable bias, 10% of the determinand concentration of the sample or $5\ \mu g\ l^{-1}$ ($0.5\ \mu g\ l^{-1}$ for cadmium), whichever is the greater; maximum tolerable total standard deviation, 5% of the determinand concentration of the sample or $2.5\ \mu g\ l^{-1}$ ($0.25\ \mu g\ l^{-1}$ for cadmium), whichever is the greater.

Work on cadmium was restarted in 1982 in response to a revision of the accuracy targets. In order to meet the more stringent requirements, it was necessary to develop and test new analytical methods. This work on cadmium will be reported later.

Analytical Quality Control

The approach followed was exactly as presented previously[2]; no attempt is made here, therefore, to explain the reasons underlying the various activities described below. The participating laboratories were as follows: Anglian Water Authority, Regional Standards Laboratory, Cambridge; Northumbrian Water Authority, Howdon Laboratory, Wallsend; North West Water Authority, Rivers Division Laboratory, Warrington; Severn-Trent Water Authority, Regional Laboratory, Finham; Southern Water Authority, Environmental Laboratory, Otterbourne; South West Water Authority, Rivers and Marine Laboratory, Exeter; Thames Water Authority, Thames Conservancy Division Laboratory, Reading; Welsh Water Authority, Chester Area Laboratory, Chester; Wessex Water Authority, Bristol Avon Divison Laboratory, Saltford; Yorkshire Water Authority, Headquarters Laboratory, Leeds; Forth RPB, Area Laboratory, Stirling. The sequence of participating laboratories in the above list does not relate to the order of numbering of laboratories in the tables.

Choice of Analytical Methods

Participating laboratories each chose a method considered capable of achieving the required accuracy; these were as

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† The names of the most recent representatives are given in the Appendix.

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follows: Laboratory 1, concentration by evaporation (20×), flame AAS, acid concentration aspirated, 1.0 M; Laboratory 2, concentration by evaporation (20×), flame AAS, acid concentration aspirated, 0.4 M; Laboratory 3, concentration by evaporation (20×), flame AAS, acid concentration aspirated 1.6 M; Laboratory 4, solvent extraction (sodium diethyldithiocarbamate - chloroform) (20×), flame AAS, organic extract evaporated and taken up in 1.3 M acid; Laboratory 5, concentration by evaporation (10×), flame AAS, acid concentration aspirated. 0.8 M; Laboratory 6, solvent extraction (sodium diethyldithiocarbamate - chloroform) (50×), flame AAS, organic extract evaporated and taken up in 1.3 M acid; Laboratory 7, concentration by evaporation (10×), flame AAS, acid concentration aspirated, 1.6 M; Laboratory 8, concentration by evaporation (10×), flame AAS (changed, January 1983, for Pb, Ni, Cd to graphite furnace AAS with direct sample injection; changed, for Cu, Zn, to flame AAS with direct sample aspiration); Laboratory 9, concentration by evaporation (10×), flame AAS, acid concentration aspirated, 1.6 M; Laboratory 10, graphite furnace AAS with direct sample injection (this method and equipment no longer used); and Laboratory 11, concentration by evaporation (20×), flame AAS, acid concentration aspirated, 3.7 M.

Within-laboratory Precision Tests

On each of 10 d, each laboratory carried out duplicate determinations on each of the following solutions: a blank, two standard solutions, a river water with metal concentrations near the lowest routinely reported for the Harmonised Monitoring Scheme, and the same river water spiked with a known concentration of each determinand.

The concentrations of the standard solutions used by each laboratory were 10 and 90%, respectively, of the upper concentration limit of the laboratory's method. These concentrations, therefore, varied from one laboratory to another (the ranges established in laboratories, in some instances, reflect the need to use the same method for the analysis of many types of sample, of which river water is only one). Each laboratory, apart from Laboratory 7, followed the agreed procedure and collected one sample of river water from a local source and used this (preserved) sample throughout the tests. All results were blank-corrected.

On completion of the tests, each laboratory analysed its results to obtain estimates of within-batch (s_w), between-batch (s_b) and total (s_t) standard deviations,[2] where $s_t = (s_w{}^2 + s_b{}^2)^{\frac{1}{2}}$. The values of s_t were compared with the appropriate target value using an *F*-test and were accepted as satisfactory provided s_t was not significantly greater ($p = 0.05$) than the appropriate target. The results of the precision tests are summarised in Tables 1–5. The results for the spiked and unspiked river water samples were used to calculate a "spiking recovery" for each metal. These are also shown in Tables 1–5. A spiking recovery that was not significantly (at the 95% confidence level) less than 95% or greater than 105% was considered acceptable.

Laboratory 7 collected a fresh sample of river water for each of the 10 d of the tests. The fact that a different river sample, of different concentrations of the metals of interest, was used on each day means that the estimate of between-batch standard deviation obtained in the analysis of variance was falsely inflated. In this sample the between-batch standard deviation for the river samples was estimated from the data submitted for the standard solutions. This estimate was combined with the measured within-batch standard deviation to arrive at an estimate of total standard deviation.

The satisfactory completion of these tests in nearly all laboratories indicated that the laboratories' precision was adequate, and the next stage of AQC work proceeded.

Comparison of Standard Solutions

At this stage in the sequential scheme of AQC it was considered appropriate to assess and control between-laboratory bias caused by differences in the concentrations of standard solutions used in each laboratory for calibration. Each laboratory was asked to analyse sufficient portions of its own standard solution and one prepared and distributed by the WRC so as to allow the detection (95% confidence level) of a difference of 2% in the concentrations. Results were returned for only five laboratories (Laboratories 1, 2, 3, 7 and 11). Of these only two instances of a difference of greater than 2% were observed—both for Laboratory 11 for nickel and zinc, 2.3 and 3.2%, respectively. The standards at Laboratory 11 were investigated and in subsequent tests of inter-laboratory bias results from this laboratory proved generally satisfactory.

Each laboratory then set up a preliminary statistical quality control chart[2] based on the analysis of a standard solution in each batch of analyses. These charts are intended to aid the continuing, long-term assessment of accuracy in each laboratory and are not discussed further here.

Tests for Inter-laboratory Bias

Portions of one standard solution and two river water samples were distributed by the WRC. The solutions were analysed once for each metal on each of 4 d. The results were then examined in relation to the agreed accuracy requirements.

To assess whether or not the bias of laboratory exceeded the target value, the following procedure was adopted. Let the mean result and its 90% confidence interval of Laboratory *i* be denoted by $x_i + L_i$. The value of the maximum possible bias of Laboratory *i* (95% confidence level) was then calculated as:

$$100(x_i + L_i - X)/X \text{ if } x_i > X$$

or

$$100(x_i - L_i - X)/X \text{ if } x_i < X$$

where *X* is the true value of the metal concentration of the distributed standard solution and the over-all mean of all laboratories' results for the two river water samples. For each test of inter-laboratory bias, statistical tests for outlying results were performed as described elsewhere.[8] Such results were detected on only two occasions (August 1981, Laboratory 8 for the determination of nickel in the low concentration river water and March 1979, Laboratory 5 for the determination of cadmium in the low concentration river water). Where an outlying result was detected it was not used in the calculation of the mean result of all laboratories, against which the inter-laboratory bias is evaluated.

The first test of inter-laboratory bias (March 1979) revealed an unacceptably large number of failures to meet the requirements. Subsequently, three further tests of inter-laboratory bias, following the same experimental design, have been undertaken. The results of the most recent test (January 1983) are given in full in Tables 6–10. The results for cadmium determination (Table 6) date from August 1981 as cadmium was not included in the test conducted in 1983 (see Accuracy Requirements).

The results of all four inter-laboratory tests are summarised in Table 11, in terms of the incidence of failure to achieve the required accuracy.

Discussion

The results indicate that many laboratories have not been achieving the accuracy specified for the Harmonised Monitoring Scheme. Control over random error was generally adequate, but systematic errors of an unacceptable size were observed for most laboratories in the period over which the tests were performed. Given that spiking recoveries were generally adequate, possible sources of systematic error include matrix interferences independent of determinand

Table 1. Results of precision tests for the determination of cadmium

	Standard solution 1					Standard solution 2					River water					"Spiked" river water					
Laboratory No.	Concentration (nominal)/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Concentration (nominal)/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Concentration found/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Concentration found/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Spiking recovery,† %
1	10.0	0.16	0.38	0.42	4.2	90.0	1.90	2.03	2.79	3.1	−0.4	0.22	N.s.	0.22	—	77.1	1.11	0.50	1.87	2.4	96.4 ± 1.2
2	5.0	0.11	0.11	0.15	3.0	50.0	0.27	N.s.‡	0.36	0.7	0.0	0.12	N.s.	0.13	—	33.6	0.12	0.33	0.36	1.1	101.0 ± 0.6
3	2.5	0.03	0.15	0.16	6.4	22.5	0.25	0.58	0.63	2.8	0.2	0.08	0.08	0.12	60	7.6	0.13	0.32	0.36	4.7	99.2 ± 2.9
4	0.5	0.02	N.s.	0.03	5.3	5.6	0.06	N.s.	0.07	1.2	0.2	0.04	N.s.	0.04	20	3.0	0.03	N.s.	0.05	1.6	98.0 ± 1.1
5	1.0	0.27	N.s.	0.27§	27.3	9.0	0.26	N.s.	0.28	3.1	0.3	0.26	N.s.	0.26§	87	5.2	0.24	N.s.	0.27	5.2§	98.4 ± 1.4
6	2.5	0.17	0.25	0.30§	12.1	22.5	0.27	0.69	0.75	3.3	0.4	0.12	0.13	0.18	45	11.8	0.22	0.23	0.32	2.7	113.8 ± 1.7
7¶	5.0	0.17	N.s.	0.19	3.9	45.0	0.29	0.47	0.56	1.2	1.0	0.18	—	0.18	18	30.8	0.47	—	0.59	1.9	93.1 ± 3.1
8	50.0	0.32	0.41	0.52	1.0	450.0	4.46	N.s.	4.65	1.0	0.8	0.10	0.10	0.14	17	300.0	2.26	N.s.	3.10	1.0	98.4 ± 0.5
9	1.0	0.16	N.s.	0.21	20.8	9.0	0.26	N.s.	0.32	3.5	0.7	0.14	0.19	0.24	34	5.4	0.18	0.26	0.32	5.4§	93.5 ± 2.1
10	1.0	0.20	N.s.	0.22	22.5	9.0	0.54	N.s.	0.70	7.8‖	0.0	0.07	0.10	0.11	—	7.6	0.37	0.51	0.63	8.2‖	94.7 ± 7.4
11	5.0	0.14	0.17	0.23	4.6	45.0	0.30	0.41	0.51	1.1	0.0	0.03	0.03	0.04	—	44.2	0.69	N.s.	0.83	1.9	98.6 ± 0.8

* s_w, s_b and s_t refer to estimates of within-batch, between-batch and total standard deviation, respectively. Target total standard deviation, 5% of determinand concentration or 0.25 µg l^{-1}, whichever is the greater.

† Spiking recovery is shown as a percentage of the expected recovery with a 90% confidence interval.

‡ N.s., not significant (*F*-test, $p = 0.05$).

§ Not significantly (*F*-test, $p = 0.05$) greater than target.

¶ Values for total standard deviation for Laboratory 7, for the river sample and spiked river sample, have been calculated from a measured within-batch standard deviation and a between-batch standard deviation inferred from standard solution data, see text for further details.

‖ Exceeds target.

Table 2. Results of precision tests for the determination of copper

	Standard solution 1					Standard solution 2					River water					"Spiked" river water					
Laboratory No.	Concentration (nominal)/ µg l^{-1}	Standard deviation*/µg l^{-1}			Relative total standard deviation, %	Concentration (nominal)/ µg l^{-1}	Standard deviation*/µg l^{-1}			Relative total standard deviation, %	Concentration found/ µg l^{-1}	Standard deviation*/µg l^{-1}			Relative total standard deviation, %	Concentration found/ µg l^{-1}	Standard deviation*/µg l^{-1}			Relative total standard deviation, %	Spiking recovery,† %
		s_w	s_b	s_t			s_w	s_b	s_t			s_w	s_b	s_t			s_w	s_b	s_t		
1	20.0	0.59	N.s.‡	0.80	4.0	180.0	1.83	2.40	3.02	1.7	2.7	0.58	N.s.	0.70	25.9	161.7	1.41	1.58	2.12	1.3	99.4 ± 0.6
2	20.0	0.71	N.s.	0.78	3.9	180.0	2.65	3.43	4.34	2.4	2.7	0.91	N.s.	0.91	33.7	136.1	3.32	N.s.	3.32	2.4	100.3 ± 0.7
3	5.0	0.27	0.33	0.43	8.6	45.0	0.55	0.91	1.06	2.3	2.7	0.37	N.s.	0.41	15.2	17.6	0.79	N.s.	1.12	6.4	99.5 ± 3.2
4	2.8	0.09	0.11	0.14	5.0	27.8	0.55	0.68	0.88	3.1	9.8	0.43	0.51	0.67	6.8	23.4	0.60	N.s.	0.75	3.2	98.4 ± 3.2
5	10.0	0.77	N.s.	0.82	8.2	90.0	0.74	1.15	1.37	1.5	7.9	0.97	N.s.	1.10	13.9	58.0	1.50	N.s.	1.61	2.8	100.2 ± 1.3
6	2.0	0.22	N.s.	0.29	14.5	20.0	0.59	0.79	0.98	4.9	2.6	0.24	N.s.	0.29	11.1	12.9	0.18	0.37	0.42	3.2	101.7 ± 2.0
7¶	5.0	0.19	0.26	0.33	6.6	45.0	0.78	0.85	1.15	2.5	10.6	0.68	—	0.72	6.8	40.4	1.25	—	1.55	3.8	94.6 ± 3.7
8**	50.0	0.42	0.96	1.04	2.1	450.0	5.47	N.s.	6.42	1.4	4.3	0.13	0.17	0.21	4.9	317.3	5.09	N.s.	7.19	2.3	104.3 ± 1.2
	(10.0)	(1.38)	(N.s.)	(1.42)	13.8	90.0	(1.48)	(N.s.)	(1.71)	1.7	(12.2)	(0.77)	(1.66)	(1.83)	(15.0)	(98.5)	(0.84)	(1.58)	(1.79)	(1.8)	(96.0 ± 2.4)
9	5.0	1.43	1.52	2.09	41.8	45.0	6.18	N.s.	6.61‖	14.7	10.0	1.00	N.s.	1.41	14.1	32.6	1.82	N.s.	1.96	6.0	90.3 ± 3.0
10	10.0	1.58	N.s.	1.90	19.0	90.0	4.53	N.s.	5.21	5.8§	2.2	1.27	N.s.	1.44	65.4	75.9	3.30	4.89	5.90	7.7‖	92.1 ± 4.5
11	5.0	0.25	N.s.	0.25	5.0	45.0	0.49	0.51	0.71	1.6	6.1	0.16	0.21	0.26	4.3	40.6	1.18	N.s.	1.19	2.9	99.2 ± 1.5

* s_w, s_b and s_t refer to estimates of within-batch, between-batch and total standard deviation, respectively. Target total standard deviation 5% of determinand concentration or 2.5 µg l^{-1}, whichever is the greater.

†–‖ See footnotes to Table 1.

** Values for Laboratory 8 shown in parentheses are for a five-batch, two-replicate test of precision using a direct aspiration flame AAS method, adopted in January 1982.

Table 3. Results of precision tests for the determination of lead

	Standard solution 1					Standard solution 2					River water					"Spiked" river water					
Laboratory No.	Concentration (nominal)/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Concentration (nominal)/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Concentration found/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Concentration found/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Spiking recovery,† %
1	20.0	2.74	N.s.‡	2.74§	13.7	180.0	2.68	3.79	4.64	2.6	−0.9	2.24	N.s.	2.27	—	154.9	1.39	3.02	3.33	2.1	97.5 ± 0.8
2	20.0	0.79	N.s.	0.81	4.0	180.0	2.56	N.s.	3.31	1.8	0.2	0.46	N.s.	0.73	27.0	135.2	1.63	N.s.	1.66	1.2	101.6 ± 0.6
3	25.0	1.12	N.s.	1.57	6.3	225.0	1.65	3.66	4.01	1.8	4.1	1.08	N.s.	1.36	33.2	76.3	1.12	2.78	3.00	3.9	100.0 ± 1.9
4	11.1	1.03	N.s.	1.11	10.0	111.1	2.28	N.s.	3.06	2.7	33.1	1.21	N.s.	1.52	4.6	87.5	2.17	N.s.	2.47	2.8	98.0 ± 1.9
5	10.0	0.97	N.s.	1.04	10.4	90.0	1.05	1.38	1.73	1.9	15.2	1.09	1.38	1.76	11.5	66.1	1.16	N.s.	1.40	2.1	102.0 ± 3.8
6	2.2	0.36	0.38	0.52	23.6	22.0	0.95	1.36	1.66	7.5	10.2	0.79	N.s.	1.00	9.8	20.9	0.58	1.40	1.52	7.3	106.9 ± 4.9
7¶	20.0	0.98	1.46	1.76	8.8	180.0	1.44	1.87	2.37	1.3	7.7	1.47	—	1.48	19.0	126.3	1.52	—	2.65	2.1	92.8 ± 1.4
8	50.0	0.99	1.38	1.70	3.4	450.0	0.99	1.38	1.70	0.4	18.5	1.00	N.s.	1.15	6.2	332.6	6.34	11.2	12.8	3.8	96.1 ± 3.1
9	5.0	0.54	0.94	1.09	21.8	45.0	2.05	N.s.	2.69§	6.0	1.6	0.84	N.s.	0.84	52.5	25.6	1.22	1.51	1.95	7.6	104.9 ± 2.3
10	10.0	2.81	N.s.	3.03§	30.3	90.0	4.16	N.s.	5.86	6.5‖	3.2	1.49	1.53	2.13	66.5	77.0	3.97	4.14	5.74	7.4‖	92.2 ± 3.5
11	5.0	0.71	N.s.	0.92	18.4	45.0	0.27	0.42	0.51	1.1	0.5	0.32	0.33	0.46	92.0	44.6	0.62	0.77	0.99	2.2	98.3 ± 1.1

* s_w, s_b and s_t refer to estimates of within-batch, between-batch and total standard deviation, respectively. Target total standard deviation 5% of determinand concentration or 2.5 µg l^{-1}, whichever is the greater.

†–‖ See footnotes to Table 1.

Table 4. Results of precision tests for the determination of nickel

	Standard solution 1					Standard solution 2					River water					"Spiked" river water					
Laboratory No.	Concentration (nominal)/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Concentration (nominal)/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Concentration found/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Concentration found/ µg l^{-1}	Standard deviation*/µg l^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Spiking recovery,† %
1	20.0	1.48	N.s.‡	1.80	9.0	180.0	4.06	N.s.	4.77	2.6	2.7	0.98	N.s.	0.98	36.3	160.5	2.35	N.s.	3.12	1.9	98.6 ± 0.8
2	20.0	0.93	N.s.	0.99	4.9	180.0	2.59	N.s.	3.28	1.8	1.1	0.83	N.s.	1.00	90.1	136.0	2.45	2.77	3.70	2.7	101.5 ± 1.3
3	10.0	0.58	0.71	0.91	9.1	90.0	0.49	1.07	1.18	1.3	0.8	0.22	0.59	0.63	78.7	27.9	0.97	N.s.	1.04	3.7	91.6 ± 1.4
4	5.6	0.26	N.s.	0.28	5.0	55.6	0.52	0.59	0.78	1.4	9.7	0.41	0.49	0.64	6.6	36.4	0.95	N.s.	1.13	3.1	96.4 ± 1.8
5	10.0	1.14	1.16	1.62	16.2	90.0	1.09	N.s.	1.17	1.3	12.8	0.59	0.94	1.11	8.7	63.0	1.05	N.s.	1.18	1.9	100.3 ± 2.4
6	2.2	0.24	0.42	0.48	21.8	22.0	0.77	1.50	1.69	7.7	2.5	0.20	0.48	0.52	20.8	14.4	0.26	0.52	0.58	4.0	119.6 ± 3.6
7¶	5.0	0.23	0.29	0.37	7.1	45.0	0.63	N.s.	0.67	1.5	10.5	0.31	—	0.33	3.1	40.1	0.64	—	0.77	1.9	93.6 ± 2.9
8	50.0	0.91	N.s.	1.02	2.0	450.0	6.54	N.s.	7.02	1.6	3.7	0.59	0.60	0.84	22.7	306.0	6.06	N.s.	6.66	2.2	100.8 ± 0.9
9	5.0	0.91	N.s.	1.05	21.0	45.0	1.81	N.s.	2.43	5.4	2.4	0.56	0.74	0.92	38.3	25.5	0.98	1.40	1.71	6.7	92.9 ± 2.8
10	20.0	3.34	N.s.	3.70‖	18.5	180.0	9.41	N.s.	13.0	7.2‖	0.8	0.95	1.74	1.98	247.5	152.3	12.2	N.s.	15.8	10.3‖	94.4 ± 4.7
11	5.0	0.58	N.s.	0.71	14.2	45.0	0.58	N.s.	0.71	1.6	0.4	0.37	N.s.	0.46	115.0	45.3	0.68	1.50	1.64	3.6	100.3 ± 4.0

* s_w, s_b and s_t refer to estimates of within-batch, between-batch and total standard deviation, respectively. Target total standard deviation, 5% of determinand concentration or 2.5 µg l^{-1}, whichever is the greater.

†, ‡, ¶ and ‖ see footnotes to Table 1.

Table 5. Results of precision tests for the determination of zinc

Laboratory No.	Standard solution 1					Standard solution 2					River water					"Spiked" river water					
	Concentration (nominal)/$\mu g\ l^{-1}$	Standard deviation*/$\mu g\ l^{-1}$			Relative total standard deviation, %	Concentration (nominal)/$\mu g\ l^{-1}$	Standard deviation*/$\mu g\ l^{-1}$			Relative total standard deviation, %	Concentration found/$\mu g\ l^{-1}$	Standard deviation*/$\mu g\ l^{-1}$			Relative total standard deviation, %	Concentration found/$\mu g\ l^{-1}$	Standard deviation*/$\mu g\ l^{-1}$			Relative total standard deviation, %	Spiking recovery,† %
		s_w	s_b	s_t			s_w	s_b	s_t			s_w	s_b	s_t			s_w	s_b	s_t		
1	10.0	0.27	N.s.‡	0.28	2.8	90.0	1.02	1.48	1.79	2.0	6.9	0.24	N.s.	0.28	4.0	84.3	1.48	N.s.	1.48	1.7	96.8 ± 0.8
2	5.0	0.74	N.s.	0.74	14.8	45.0	1.22	N.s.	1.22	2.7	3.3	0.86	N.s.	0.95	28.8	36.3	0.93	N.s.	1.29	3.5	99.1 ± 1.5
3	2.5	0.07	0.24	0.25	10.0	22.5	0.21	0.37	0.43	1.9	12.2	0.12	0.78	0.79	6.4	19.6	0.20	0.71	0.74	3.8	99.6 ± 4.2
4	11.1	0.71	N.s.	0.72	6.5	111.1	1.06	N.s.	1.18	1.0	24.1	1.22	N.s.	1.63	6.8	77.4	1.66	1.84	2.48	3.2	95.9 ± 1.9
5	10.0	0.77	0.84	1.14	11.4	90.0	0.74	2.59	2.69	3.0	76.7	1.32	2.46	2.79	3.6	120.0	1.30	1.52	2.00	1.7	92.0 ± 3.4
6	2.2	0.68	N.s.	0.71	32.3	22.0	1.21	N.s.	1.33	5.9	14.3	0.41	1.00	1.09	7.6	25.1	0.79	2.03	2.12	8.4	108.4 ± 8.0
7¶	10.0	0.56	N.s.	0.71	7.1	90.0	0.43	1.54	1.60	1.8	18.6	1.71	—	1.75	9.4	78.8	1.80	—	2.38	3.0	95.2 ± 1.6
8**	50.0	0.48	0.78	0.91	1.8	450.0	3.27	3.92	5.11	1.1	324.5	2.06	3.72	4.25	1.3	968.1	4.18	10.9	11.7	1.2	109.2 ± 1.2
	(20.0)	(0.00)	(1.14)	(1.14)	(6.5)	(180.0)	(0.55)	(2.60)	(2.66)	(1.4)	(25.8)	(1.26)	(N.s.)	(2.00)	(7.7)	(198.8)	(0.45)	(1.79)	(1.85)	(0.43)	(92.2 ± 2.5)
9	20.0	4.08	N.s.	5.30‖	26.5	180.0	6.95	7.68	10.4	5.8§	60.3	3.15	N.s.	3.73	6.2§	151.7	3.74	7.30	8.21	5.4§	91.3 ± 4.2
10	50.0	5.22	6.99	8.72	17.4‖	450.0	16.3	N.s.	22.0	4.9	24.8	6.47	N.s.	8.64‖	34.8	394.7	13.3	N.s.	15.6	3.9	92.5 ± 1.8
11	5.0	0.22	0.34	0.41	8.2	45.0	0.39	0.53	0.66	1.5	5.9	0.19	0.32	0.38	3.3	44.8	0.74	N.s.	0.88	2.0	97.8 ± 2.1

* s_w, s_b and s_t refer to estimates of within-batch, between-batch and total standard deviation, respectively. Target total standard deviation, 5% of determinand concentration or 2.5 $\mu g\ l^{-1}$, whichever is the greater.

†–‖ See footnotes to Table 1.

** See footnote to Table 2.

Table 6. Results of the most recent test of inter-laboratory bias (August 1981) for cadmium. All results are in μg l^{-1} unless otherwise indicated

	Low concentration river water				Standard solution				High concentration river water			
Laboratory No.	Mean result	Standard deviation	Difference from mean of all results	Maximum possible bias*	Mean result	Standard deviation	Difference from WRC value	Maximum possible bias*	Mean result	Standard deviation	Difference from mean of all results, %	Maximum possible bias,* %
1	2.16	0.09	−0.03	−0.13	4.16	0.14	0.16	0.33	5.52	0.25	6.24	11.82†
2	2.33	0.13	0.13	0.28	4.15	0.09	0.15	0.26	5.44	0.06	4.74	6.16
3	2.63	0.25	0.43	0.73†	4.13	0.25	0.13	0.42	5.13	0.25	−1.28	−6.95
4	2.14	0.06	−0.05	−0.12	3.98	0.06	−0.02	−0.09	5.33	0.09	2.57	4.70
5	2.54	0.23	0.34	0.61†	4.68	0.48	0.68†	1.25†	5.76	0.36	11.00†	19.08†
6	2.35	0.17	0.16	0.36	4.48	0.26	0.48	0.79†	5.53	0.17	6.42	10.29†
7	2.08	0.26	−0.12	−0.43	3.65	0.24	−0.35	−0.63†	4.83	0.56	−7.06	−19.66†
8	2.13	0.10	−0.07	−0.18	3.90	0.18	−0.10	−0.31	5.23	0.19	0.64	4.93
9	1.80	0.14	−0.39	−0.56†	3.13	0.33	−0.87†	−1.26†	4.23	0.29	−18.62†	−25.13†
10‡												
11	1.80	0.08	−0.39	−0.49	3.84	0.02	−0.16	−0.19	4.95	0.06	−4.65	−5.96
Mean of all laboratories ..	2.19				Mean of all laboratories	4.01			Mean of all laboratories	5.19		
WRC (analysed) value ..	2.1				WRC value (prepared concn.)	4.0			WRC (analysed) value ..	5.3		

* The target for maximum possible bias is 10% of the concentration of the sample or 0.5 μg l^{-1}, whichever is the greater. The values given for maximum possible bias are quoted at a confidence level of 95%.

† Exceeds target.

‡ Although Laboratory 10 did analyse the solutions distributed in this test, its results are not presented because no evidence was available to verify that the precision of the method used was adequate.

Table 7. Results of the most recent test of inter-laboratory bias (January 1983) for copper. All results are in μg/l^{-1} unless otherwise indicated

	Low concentration river water				Standard solution				High concentration river water			
Laboratory No.	Mean result	Standard deviation	Difference from mean of all results	Maximum possible bias*	Mean result	Standard deviation	Difference from WRC value	Maximum possible bias*	Mean result	Standard deviation	Difference from mean of all results, %	Maximum possible bias,* %
1	25.11	0.86	0.36	1.37	39.33	0.71	−0.67	−1.50	55.65	1.48	2.44	5.65
2	24.25	0.65	0.49	−1.25	38.88	0.85	−1.13	−2.13	54.63	0.85	0.55	2.40
3	23.63	1.60	−1.12	−3.00	36.75	3.12	−3.25	−6.92†	49.75	4.37	−8.42	−17.88†
4	20.85	0.45	−3.89	−4.42	31.60	0.82	−8.40†	−9.37†	48.48	0.86	−10.77†	−12.63†
5	26.63	1.50	1.88	3.65	38.48	0.64	−1.52	−2.28	56.45	0.53	3.91	5.06
6‡	27.17	0.46	2.43	2.97	36.37	0.79	−3.62	−4.55	55.37	1.45	1.92	5.07
7§												
8	26.30	1.61	1.56	3.45	38.40	0.88	−1.60	−2.64	58.70	1.15	8.05	10.54†
9	25.45	0.58	0.71	1.38	41.20	1.26	1.20	−2.68	56.45	1.73	3.91	7.66
10‡												
11	25.75	0.50	1.01	1.59	36.75	0.96	−3.25	−4.38	54.50	3.00	0.32	6.82
Mean of all laboratories ..	24.74				Mean of all laboratories	37.67			Mean of all laboratories	54.33		
WRC (analysed) value ..	24.6				WRC value (prepared concn.)	40			WRC (analysed) value ..	58.0		

* The target for maximum possible bias is 10% of the concentration of the sample or 5 μg l^{-1}, whichever is the greater. The values given for maximum possible bias are quoted at a confidence level of 95%.

† Exceeds target.

‡ Although Laboratories 6 and 10 did analyse the solutions distributed in this test, their original results are not presented because no evidence was available to verify that the precision of the method used was adequate. Laboratory 6 subsequently performed analyses using the method for which precision tests had been performed. These results are shown but have not been used in the calculation of the mean result of laboratories owing to their late receipt.

§ Laboratory 7 did not report results for this test.

concentration, inability to detect all forms of the determinand and biased calibration.

An examination of Table 11 reveals two interesting features. Firstly, it is evident that changes have occurred in the performance of several of the laboratories during the period of the tests. Secondly, on any given test, a laboratory showing a high proportion of failures on one metal also tends to perform badly for other metals. This suggests that sources of systematic error are common to all the metals determined. Examination of the results in Tables 6–10 reveals a tendency for errors for a given laboratory to be in the same direction. This could arise from biased calibration (*e.g.*, Laboratory 3), but other factors, such as low recovery of metal from real samples during solvent extraction or precipitation during evaporation, could be responsible for such results.

The reasons for the failure of laboratories to meet the accuracy requirements cannot be known with certainty, but the following contributory factors could be suggested. (1) Lack of proven analytical methods. At the outset of the work the performance of even the few methods available had not been adequately tested for use with the wide variety of sample types that were to be encountered. (2) Systematic error arising from biased calibration. The failure of all laboratories to check their concentrated standard solutions used for calibration is likely to have contributed to the apparent lack of control of systematic error. (3) The long period over which the work had been undertaken and the response to the results of the tests. Inevitable long-term changes that occur in any laboratory (in staff, instrumentation and in location and laboratory facilities) are likely to cause a disruption in the quality of results from

ห้องสมุด [illegible]

Table 8. Results of the most recent test of inter-laboratory bias (January 1983) for lead. All results are in µg l⁻¹ unless otherwise indicated

	Low concentration river water				Standard solution				High concentration river water			
Laboratory No.	Mean result	Standard deviation	Difference from mean of all results	Maximum possible bias*	Mean result	Standard deviation	Difference from WRC value	Maximum possible bias*	Mean result	Standard deviation	Difference from mean of all results, %	Maximum possible bias,* %
1	26.52	0.58	2.30	2.98	39.31	1.11	−0.69	−2.00	55.62	0.75	8.46	10.19†
2	24.50	0.71	0.28	1.11	36.88	1.03	−3.13	−4.34	51.63	0.25	0.68	1.26
3	21.63	1.89	−2.59	−4.81	36.25	2.20	−3.75	−6.34†	46.13	4.01	−10.04†	−19.30
4	23.63	0.95	−0.59	−1.70	34.63	3.35	−5.38†	−9.32†	52.88	0.85	3.12	5.09
5	23.55	0.24	−0.67	−0.95	37.23	0.13	−2.77	−2.92	50.53	0.47	−1.46	−2.55
6‡	27.90	1.53	3.68	5.47†	42.42	0.89	2.42	3.47	52.47	0.61	2.32	3.72
7§												
8‡												
9	26.20	0.96	1.98	3.10	42.70	0.50	2.70	3.29	56.20	1.50	9.59	13.06†
10‡												
11	23.50	4.43	−0.72	−5.94†	33.75	2.75	−6.25†	−9.49†	46.00	4.08	−10.30†	−19.74†

Mean of all laboratories ..	24.22	Mean of all laboratories	37.89	Mean of all laboratories	51.28
WRC (analysed) value ..	25.5	WRC value (prepared concn.)	40	WRC (analysed) value ..	55.1

* The target for maximum possible bias is 10% of the concentration of the sample or 5 µg l⁻¹, whichever is the greater. The values given for maximum possible bias are quoted at a confidence level of 95%.

† Exceeds target.

‡ Although Laboratories 6, 8 and 10 did analyse the solutions distributed in this test, their original results are not presented because no evidence was available to verify that the precision of the method used was adequate. Laboratory 6 subsequently performed analysis using the method for which precision tests had been performed. These results are shown but have not been used in the calculation of the mean result of laboratories owing to their late receipt.

§ Laboratory 7 did not report results for this test.

Table 9. Results of the most recent test of inter-laboratory bias (January 1983) for nickel. All results in µg l⁻¹ unless otherwise indicated

	Low concentration river water				Standard solution				High concentration river water			
Laboratory No.	Mean result	Standard deviation	Difference from mean of all results	Maximum possible bias*	Mean result	Standard deviation	Difference from WRC value	Maximum possible bias*	Mean result	Standard deviation	Difference from mean of all results, %	Maximum possible bias,* %
1	24.62	1.33	1.86	3.42	39.92	0.85	−0.08	−1.08	63.49	0.73	7.23	8.95
2	26.13	1.80	3.37	5.48†	38.25	2.25	−1.75	−4.40	66.88	3.92	12.95†	20.63†
3	20.63	1.18	−2.13	−3.52	34.13	1.80	−5.88†	−7.99†	51.50	3.66	−13.02†	−20.18†
4	21.00	0.96	−1.76	−2.89	31.75	2.65	−8.25†	−11.36†	57.00	1.50	−3.73	−6.66
5	22.48	0.30	−0.28	−0.63	37.30	1.49	−2.70	−4.45	59.18	0.72	−0.05	−1.46
6‡	25.07	1.17	(2.31)	3.70	38.47	2.63	−1.52	−4.61	70.0	2.59	(18.22)†	23.29†
7§												
8‡												
9	21.95	0.82	−0.81	−1.79	37.95	0.82	−2.05	−3.01	60.20	0.96	1.67	3.55
10‡												
11	22.50	1.29	−0.26	−1.78	35.75	1.26	−4.25	−5.73†	56.25	1.71	−5.03	−8.37

Mean of all laboratories ..	22.76	Mean of all laboratories	37.26	Mean of all laboratories	59.21
WRC (analysed) value ..	35.6	WRC value (prepared concn.)	40.00	WRC (analysed) value ..	66.0

* The target for maximum possible bias is 10% of the concentration of the sample or 5 µg l⁻¹, whichever is the greater. The values given for maximum possible bias are quoted at a confidence level of 95%.

† Exceeds target.

‡ Although Laboratories 6, 8 and 10 did analyse the solutions distributed in this test, their original results are not presented because no evidence was available to verify that the precision of the method used was adequate. Laboratory 6 subsequently performed analysis using the method for which precision tests had been performed. These results are shown but have not been used in the calculation of the mean result of laboratories owing to their late receipt.

even the most well established methods. For methods that are being developed or those which have been found to require improvement, progress may be slow or non-existent unless the method is established over a period that is short in relation to this cycle of laboratory reorganisations. The absence of marked improvement in analytical accuracy also reflects the way in which many laboratories retained the same methods, despite poor initial performance. (4) Environmental factors leading to sample contamination. The main impact of contamination during analysis is likely to be on precision rather than on systematic error. The results of these tests suggest that, whilst some samples may have become contaminated, sample contamination was not a major problem. In confirmation of this, the blank results obtained in the 10-d precision tests do not indicate contamination as a major problem. Excluding the results of Laboratory 10, the results of blank determinations are both adequately precise and of low value. Mean blank values were 0.3 µg l^{-1} for cadmium, 1.2 µg l^{-1} for copper, nickel and lead and 3.7 µg l^{-1} for zinc.

Accepting that laboratories have not attained the required standard of analytical accuracy, what requirements for accuracy could have been achieved? Table 12 presents a summary of the results of the four tests of inter-laboratory bias in a similar form to that used in Table 11. Here, however, the criterion for maximum tolerable systematic error has been set at twice that required for the Harmonised Monitoring Scheme

Table 10. Results of the most recent test of inter-laboratory bias (January 1983) for zinc. All results in µg l^{-1} unless otherwise indicated

	Low concentration river water				Standard solution				High concentration river water			
Laboratory No.	Mean result	Standard deviation	Difference from mean of all results	Maximum possible bias*	Mean result	Standard deviation	Difference from WRC value	Maximum possible bias*	Mean result	Standard deviation	Difference from mean of all results, %	Maximum possible bias,* %
1	20.51	0.09	0.36	0.46	38.92	0.94	−1.08	−2.19	52.59	0.88	4.53	6.58
2	20.25	1.32	0.10	1.66	34.75	0.65	−5.25†	−6.01†	50.00	0.41	−0.61	−1.57
3	18.25	1.00	−1.90	−3.07	36.00	1.76	−4.00	−6.07†	45.88	1.60	−8.81	−12.55†
4	19.13	2.66	−1.02	−4.15	33.88	0.63	−6.13†	−6.87†	46.88	3.42	−6.82	−14.83†
5	18.98	0.33	−1.17	−1.56	39.90	0.54	−0.10	−0.73	50.23	0.17	−0.16	−0.56
6‡	25.55	0.99	5.40†	6.57†	41.20	0.68	1.20	2.00	54.57	0.40	8.48	9.42
7§												
8	23.08	0.90	2.93	3.99	38.85	0.95	−1.15	−2.27	54.65	0.39	8.63	9.54
9	19.75	0.00	−0.40	−0.40	39.50	0.96	−0.50	−1.63	51.25	0.58	1.87	3.22
10‡												
11	21.25	2.63	1.10	4.20	36.50	1.73	−3.50	−5.54†	51.00	3.27	1.38	9.01
Mean of all laboratories ..	20.15				Mean of all laboratories .	37.29			Mean of all laboratories	50.31		
WRC (analysed) value ..	20.9				WRC value (prepared concn.)	40			WRC (analysed) value ..	55.2		

* The target for maximum possible bias is 10% of the concentration of the sample or 5 µg l^{-1}, whichever is the greater. The values given for maximum possible bias are quoted at a confidence level of 95%.

† Exceeds target.

‡ Although Laboratories 6 and 10 did analyse the solutions distributed in this test, their original results are not presented because no evidence was available to verify that the precision of the method used was adequate. Laboratory 6 subsequently performed analysis using the method for which precision tests had been performed. These results are shown but have not been used in the calculation of the mean result of laboratories owing to their late receipt.

§ Laboratory 7 did not report results for this test.

Table 11. Instances of failure of laboratories to meet accuracy requirements in tests of inter-laboratory bias. Scores shown represent instances where the result of the analysis of a solution failed to meet requirement for accuracy*,†

	Date of test																							
	March 1979						November 1980						August 1981						January 1983					
Laboratory No.	Cd	Cu	Pb	Ni	Zn	Total	Cd	Cu	Pb	Ni	Zn	Total	Cd	Cu	Pb	Ni	Zn	Total	Cd	Cu	Pb	Ni	Zn	Total
1	1	0	0	2	1	4	0	0	0	0	0	0	1	0	0	0	0	1	–	0	1	0	0	1
2	2	0	0	1	1	4	0	0	0	0	0	0	0	0	0	0	1	1	–	0	0	2	1	3
3	1	0	0	1	3	5	0	0	1	0	0	1	1	0	0	0	0	1	–	2	2	2	2	8
4	2	2	2	–	1	7	0	0	0	0	0	0	0	0	1	0	0	1	–	2	1	1	2	6
5	3	1	0	0	1	5	2	0	0	0	0	2	3	1	1	0	2	7	–	0	0	0	0	0
6	2	1	0	2	1	6	3	2	1	1	2	9	2	3	1	0	2	8	–	0	1	1	1	3
7	0	0	0	0	1	1	0	0	2	0	2	4	2	3	3	1	3	12	–	–	–	–	–	–
8	2	2	1	1	2	8	2	1	3	1	3	10	0	3	0	3	2	8	–	1	–	–	0	1
9	1	2	0	0	1	4	1	2	0	0	0	3	3	0	0	0	0	3	–	0	1	0	0	1
10	2	3	3	3	3	14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	2	1	0	1	2	6	0	0	0	0	0	0	0	0	0	0	0	0	–	0	3	1	1	5
Total failures per metal ..	18	12	6	11	17		8	5	7	2	7		12	10	6	4	10		–	5	9	7	7	
Grand total‡ ..			64	(50)					29						42						28			
Failure, ‡ % ..			39	(34)					19						28						27			

* In each test of inter-laboratory bias laboratories carried out determinations of each metal on each of three samples (2 river waters and a dilute standard solution). Scores of failure in each test for a given laboratory and given metal are, therefore out of a maximum of 3.

† The requirement for accuracy in these tests was that maximum possible bias should not be larger than 5 µg l^{-1} (0.5 µg l^{-1} for Cd) or 10% of determinand concentration, whichever is the greater.

‡ Grand total and percentage failure values in parentheses are calculated omitting the results of Laboratory 10.

[10 µg l^{-1} (1 µg l^{-1} for Cd) or 20% of the determinand concentration, whichever is the greater]. Judged against this more lax requirement, the performance of laboratories would be regarded as broadly satisfactory.

It is likely that the only way in which the accuracy of analytical results could be improved so as to meet the existing requirements of the Harmonised Monitoring Scheme would be to review and re-evaluate the methods used. This would probably involve many laboratories adopting different methods (*e.g.*, carbon furnace AAS with, possibly, some form of pre-treatment such as solvent extraction) for the determination of these metals. It is not possible, within the lifetime of the contract for the co-ordination of AQC for the Scheme, to embark on such a programme of work.

Conclusions

Many participating laboratories did not achieve the required accuracy for the determination of trace metals. Random error was generally controlled to an adequate degree but systematic errors of an unacceptable size were observed for most laboratories in the period during which tests were performed.

Work for the Harmonised Monitoring Scheme on other determinands has supported the validity of the approach to AQC adopted by the AQC (HM) Committee. It is considered that this approach is not inappropriate for the determination of these trace metals. Indeed, the sequential approach to AQC is designed to allow the identification of the causes of analytical error and is more likely to be value for the more

Table 12. Instances of failure of laboratories to meet accuracy requirements in tests of inter-laboratory bias judged against targets less stringent than those specified for the Harmonised Monitoring Scheme. Scores shown represent instances where the result of analysis of a solution failed to meet requirement for accuracy*,†

	Date of test																							
	March 1979						November 1980						August 1981						January 1983					
Laboratory No.	Cd	Cu	Pb	Ni	Zn	Total	Cd	Cu	Pb	Ni	Zn	Total	Cd	Cu	Pb	Ni	Zn	Total	Cd	Cu	Pb	Ni	Zn	Total
1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	–	0	0	0	0	0
2	1	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	–	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	0	0	1	0	1
4	1	0	0	–	0	1	0	0	0	0	0	0	0	0	0	0	0	0	–	0	0	1	0	1
5	2	1	0	0	0	3	0	0	0	0	0	0	1	0	0	0	1	2	–	0	0	0	0	0
6	0	0	0	1	0	1	1	1	1	1	0	4	0	0	0	0	0	0	–	0	0	1	0	1
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	4	–	–	–	–	–	–
8	2	0	0	0	1	3	1	0	1	0	1	3	0	0	0	2	0	2	–	0	–	–	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	–	0	0	0	0	0
10	1	1	1	2	3	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	0	0	0	0	0
Total failures per metal ..	7	2	1	3	6		2	1	2	1	1		3	0	1	3	3		–	0	0	3	0	
Grand total‡ ..			19	(11)					7						10						3			
Failures, % ..			12	(7)					5						7						3			

* In each test of inter-laboratory bias laboratories made determinations of each metal on each of three samples (2 river waters and a dilute standard solution). Scores of failure in each test for a given laboratory and given metal are therefore out of a maximum of 3.

† The requirement for accuracy used in compiling this table was that maximum possible bias should not be larger than 10 μg l^{-1} (1 μg l^{-1} for Cd) or 20% of determinand concentration, whichever is the greater. The accuracy requirement adopted for the Harmonised Monitoring Scheme requires maximum possible bias to be not greater than half of these values, *i.e.*, 5 μg l^{-1} (0.5 for Cd) or 10%.

‡ Grand total and percentage failure values in parentheses are calculated omitting the results of Laboratory 10.

problematic determinations. In this instance, however, problems resulting from a lack of suitable analytical methods were not resolved in laboratories, either by method development or by the adoption of methods of better performance. The failure of some laboratories to check their stock standard solutions is also likely to have contributed to the lack of control over systematic error.

The standard of accuracy that could have been achieved for the determination of these trace metals was as follows: maximum tolerable bias, 10 μg l^{-1} (1 μg l^{-1} for cadmium) or 20% of the determinand concentration of the sample, whichever is the greater; and maximum tolerable total standard deviation, 5 μg l^{-1} (0.5 μg l^{-1} for cadmium) or 5% of the determinand concentration of the sample, whichever is the greater.

Although the analytical work reported here was performed in Regional Water Authority and River Purification Board laboratories, the co-ordination of the work was carried out by the Water Research Centre under contract to the Department of the Environment, whose permission to publish is acknowledged.

Appendix

The following are current members of the Analytical Quality Control (Harmonised Monitoring) Committee: Mr. L. R. Pittwell and Mr. N. Taylor (Department of the Environment); Dr. D. T. E. Hunt and Mr. M. J. Gardner (Water Research Centre); Mr. N. Loaring (Southern Water Authority); Mr. R. Lamb (Welsh Water Authority); Mr. D. Best (Yorkshire Water Authority); Dr. B. T. Croll (Anglian Water Authority); Mr. D. V. Hopkin (Thames Water Authority); Mr. A. G. Poole (Wessex Water Authority); Mr. B. Dale (South West Water Authority); Mr. T. D. Macdonald (Scottish Development Department); Mr. A. Hollington (North West Water Authority); Mr. W. Wollers (Northumbrian Water Authority); Mr. K. Bamford (Severn-Trent Water Authority); and Mr. I. R. M. Black (Forth River Purification Board).

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Effect of Surfactants on the Determination of Nitrate in Stream Waters by Using a Nitrate Ion-selective Electrode

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Nitrate-nitrogen in stream waters containing anionic surfactants was determined with a nitrate ion-selective electrode. The results with 22 stream waters agreed well with those obtained by spectrophotometry. Nitrite, hydrogen carbonate and chloride interference were removed by the addition of sulphamic acid, sulphuric acid and silver sulphate, respectively. The interference from anionic surfactants was no problem provided its concentration was lower than 1 mg l^{-1} as dodecylbenzenesulphonate (DBS). The error caused by DBS depends on its concentration and can be mostly eliminated by the addition of a cationic surfactant such as cetyltrimethylammonium bromide. An anomalous response behaviour in the presence of some cationic surfactants was observed and is discussed in relation to their extractability as nitrate salts.

Keywords: *Nitrate ion-selective electrode; nitrate determination; stream waters; anionic surfactants; cationic surfactants*

One of the most attractive methods for the direct analysis of natural waters is a nitrate ion-selective electrode, which can be used for the simple and rapid determination of nitrate in place of the conventional time-consuming spectrophotometric procedure. More than 20 papers have been published that deal with the application of nitrate ion-selective electrodes in water analysis.[1] Simeonov *et al.*[2] determined 1.1–2.9 mg l^{-1} of nitrate-nitrogen in lake water by several potentiometric methods and compared the results. Tsuzuki and Hikosaka[3] analysed river waters containing 0.8–4.7 mg l^{-1} of nitrate-nitrogen after pre-treatment.

In Shiga Prefecture is Lake Biwa, which is the largest lake in Japan and into which over 100 streams flow. Some of the streams are polluted by domestic effluents, *e.g.*, anionic surfactants. Although many studies have been made of the determination of nitrate in environmental samples by using a nitrate ion-selective electrode, few have dealt with the determination of nitrate in stream waters containing anionic surfactants. The development of a simple and reliable method for the determination of nitrate in polluted stream waters is essential because nitrate is one of the causes of eutrophication, and the prevention of the eutrophication of Lake Biwa is one of the most urgent environmental problems in Japan.

The aim of this work was to evaluate a nitrate ion-selective electrode for stream water analysis. The interferences due to anionic surfactants and their elimination by the addition of cationic surfactants were examined. During the study, an anomalous response behaviour was observed in the presence of some cationic surfactants when the concentration of nitrate was sufficiently high.

Experimental

Apparatus

An Orion 93–07 nitrate ion-selective electrode and an Orion 90–02 double-junction reference electrode were used. The outer filling solution of the reference electrode was 0.4 M ammonium sulphate solution. The potentials at room temperature (20 ± 1 °C) were measured to 0.1 mV with an Orion Ionalyzer Model 701A and recorded with a pen recorder (Rikadenki Model R-10).

Reagents

Potassium nitrate, dried for 2 h at 110 °C, was used to prepare a standard solution. Analytical-reagent grade sodium dodecylbenzenesulphonate (DBS) (Nakarai Chemicals) was used as an anionic surfactant. The cationic surfactants used were decyltrimethylammonium bromide, dodecyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, stearyltrimethylammonium chloride, cetylpyridinium bromide, cetylbenzyldimethylammonium chloride (Tokyo Kasei Chemicals), cetyltrimethylammonium bromide (CTMA) (Nakarai Chemicals) and tetradecylbenzyldimethylammonium chloride (Dojin Chemicals), and they were used without further purification. Their solutions were prepared using distilled water.

Analytical Procedure

To 50.0 ml of sample solution were added 1 ml of 2 M ammonium sulphate solution and 0.5 ml of 0.2 N sulphuric acid that contained 2 g l^{-1} of sulphamic acid. After allowing the mixture to stand for 1 h, which was sufficient for the complete decomposition of nitrite by sulphamic acid, 2 ml of 1.76×10^{-2} M silver sulphate solution were added[4] and the electrode potential 1 min after immersion of the electrode pair was measured. This procedure brought the pH of the sample solution to approximately 3, which served to eliminate the interference from hydrogen carbonate and also those due to up to 50 mg l^{-1} of chloride and 2.9 mg l^{-1} of nitrite. A calibration graph was constructed using 0.1, 0.2, 0.3, 0.5, 1.0 and 10.0 mg l^{-1} standard nitrate solution after the same pre-treatment as for the samples.

For samples containing anionic surfactants, a cationic surfactant, dissolved in the sulphuric acid containing sulphamic acid, was added to eliminate the interference.

Nitrate was also determined by the spectrophotometric procedure, in which nitrate was reduced to nitrite with zinc powder in hydrochloric acid and reacted with GR reagent (Griess - Romijn reagent, a mixture of sulphanilamide and α-naphthylamine).[5]

Anionic surfactants in stream water were measured as methylene blue active substances (MBAS).[6]

Results and Discussion

Stream Water Analysis

Twenty-two stream waters flowing into Lake Biwa and Seta River were analysed by the procedure described above. The calibration graph in the absence of cationic surfactants was linear (55.1 mV decade^{-1}) down to 0.5 mg l^{-1} of nitrate-nitrogen but graphical evaluation was necessary below this concentration as shown in graph 1 of Fig. 1. A stable potential to within 0.1 mV was attained in less than 1 min unless the

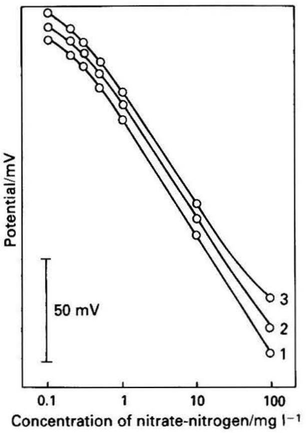

Fig. 1. Calibration graphs for nitrate-nitrogen: 1, in the absence of cationic surfactants; 2, in the presence of CTMA; and 3, in the presence of tetradecylbenzyldimethylammonium. Cationic surfactant added in an equimolar amount to 10 mg l^{-1} of Na DBS. Each graph is displaced by 6.25 mV at 0.1 mg l^{-1}

sample was contaminated with anionic surfactants. The average values and the concentration ranges of nitrate, nitrite and chloride in the stream waters were 1.39 mg l^{-1} (0.284–4.26 mg l^{-1}), 0.069 mg l^{-1} (0.004–0.301 mg l^{-1}) and 12.3 mg l^{-1} (4.34–58.4 mg l^{-1}), respectively. The agreement with the spectrophotometric results was fairly good as judged from the correlation coefficient of 0.990 and the correlation equation, $y = 0.082 + 0.945x$, where y is the value obtained by spectrophotometry and x that obtained by potentiometry. The average value and the concentration range of MBAS was 0.439 mg l^{-1} (0.031–1.32 mg l^{-1}).

The selectivity coefficient of DBS calculated from measurement at 10 mg l^{-1} was reported to be larger than 10^3, implying that the presence of several milligrams per litre of anionic surfactants made the determination impossible.[7] However, our results implied that the interference from MBAS in such a concentration range caused an insignificant error, as with inorganic interferents such as nitrite, chloride and hydrogen carbonate. The reason why the interference of DBS was smaller than expected is that the selectivity coefficient of DBS was much smaller than the reported value when its concentration was around 1 mg l^{-1}. For example, the selectivity coefficient of DBS estimated at 1 mg l^{-1} was about 5 (concentration of nitrate-nitrogen = 1 mg l^{-1}). Another reason is that the potential after 1 min, by which time the interference was still not significant, was used for the evaluation of nitrate. Subsequent work was concentrated on the interference of DBS and its elimination with cationic surfactants.

Suppression of Interference from DBS by CTMA

The interference of anionic surfactants in the determination of nitrate in environmental samples and its elimination were investigated by Takehara *et al.*[7] They used a highly porous polymer bead packed column to remove anionic surfactants or added higher alkylamines such as laurylamine to precipitate anionic surfactants from the sample solution acidified to pH 3. Although the interference was reported to be eliminated effectively by these methods, they seemed to be still inconvenient and time consuming because, for example, agitation of the sample solution for 10 min was recommended after the addition of laurylamine. We examined the effect of the addition of cationic surfactants to eliminate the interference of DBS. On the basis of the results reported later, we recommend the addition of CTMA. The calibration graph in the presence of CTMA is shown as graph 2 in Fig. 1. Little effect was observed with CTMA up to 10 mg l^{-1} of nitrate-nitrogen. At 100 mg l^{-1} of nitrate-nitrogen it took 3–5 min to obtain a stable potential reading, and this equilibrium potential was several millivolts higher than that in the absence of CTMA. This anomalous behaviour of cationic surfactants has not previously been reported and will be discussed later.

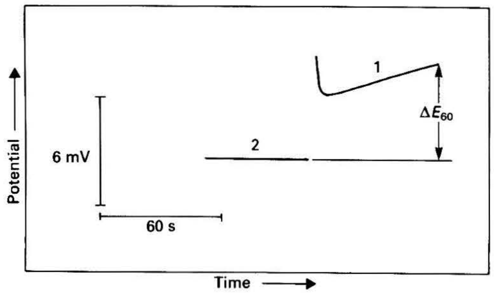

Fig. 2. Potential - response graph for 1 mg l^{-1} of nitrate-nitrogen: 1, in the presence of CTMA; and 2, in the presence of both CTMA and DBS. Concentration of DBS, 10 mg l^{-1}; CTMA added in an equimolar amount to 10 mg l^{-1} of Na DBS

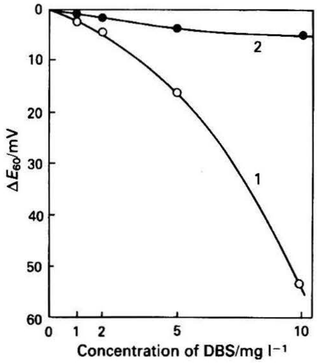

Fig. 3. Effect of DBS on the response of nitrate ion-selective electrode: 1, in the absence of CTMA; and 2, in the presence of CTMA added in an equimolar amount to 10 mg l^{-1} of Na DBS. Concentration of nitrate-nitrogen, 1 mg l^{-1}

Fig. 2 shows the potential - response graph for standard nitrate-nitrogen and a sample solution containing DBS. The potential changed positively and rapidly just after the immersion of the electrode pair and about 10 s later it showed a gradual decrease to a more negative value. Great care should be taken with regard to the shift of the standard electrode potential of the nitrate ion-selective electrode after it has been immersed in a solution containing anionic surfactants. We stopped the measurement 1 min after the immersion and the potential difference, ΔE_{60}, shown in Fig. 2 was used as a measure of the supression effect by cationic surfactants.

Fig. 3 demonstrates the suppression effect of CTMA on the interference of DBS. When the concentration of DBS was as low as 1 mg l^{-1}, its interference in the nitrate determination was fairly small, but it showed extreme interference at 10 mg l^{-1}, as already pointed out.[7] In contrast, the interference of DBS was mostly removed by the addition of CTMA. However, a small interfering effect of DBS remained even in the presence of a 10-fold excess of CTMA. The error still remained after the addition of CTMA was evaluated, as shown in Table 1. The error was independent of the concentration of nitrate-nitrogen when it was higher than 1 mg l^{-1}, but became significant when it was as low as 0.1 mg l^{-1}.

Effect of Other Cationic Surfactants

The effect of cationic surfactants can be divided into two parts, the suppression effect due to the interference of DBS and the interfering effect on the response of the nitrate ion-selective electrode, especially at high concentrations of nitrate. In the latter instance no anionic surfactant was present in solution.

The suppression effect of cationic surfactants is summarised in Table 2. In the alkyltrimethylammonium series, the interference of DBS decreased with increasing carbon chain length. Although stearyltrimethylammonium was the most effective of the cations tested, its low solubility in water prevented its convenient use. Other alkylpyridinium and alkyldimethylbenzylammonium cations were also as effective as CTMA as far as the suppression of the interference from DBS was concerned. This effect may be related to the association between DBS and the cationic surfactant in solution. Goto *et al.*[8] measured the association constant with a ferron (7-iodoquinolin-8-ol-5-sulphonic acid) - cationic surfactant complex system. Their association constants with the cationic surfactants listed in Table 2 correspond to the variation of ΔE_{60}, at least qualitatively: the larger the logarithmic association constants, the smaller is ΔE_{60}, *i.e.*, the more effective it becomes.

The anomalous response of nitrate-nitrogen at 100 mg l^{-1} that occurred with CTMA was also observed more significantly with alkylpyridinium and alkylbenzyldimethylammonium cations. In Fig. 1, the calibration graph in the presence of tetradecylbenzyldimethylammonium is shown as an example (see graph 3). The response of nitrate-nitrogen at 100 mg l^{-1} with these cations was so slow that it took more than 5 min to reach a steady potential reading. With cetylbenzyldimethylammonium, a slow response was observed even in a solution of 10 mg l^{-1} of nitrate-nitrogen. The sequence of the effect was cetylbenzyldimethylammonium > tetradecylbenzyldimethylammonium ≈ cetylpyridinium > stearyltrimethylammonium ≈ cetyltrimethylammonium. Other alkyltrimethylammonium ions tested showed no effect.

Table 1. Effect of CTMA on the suppression of interference from DBS. CTMA was added in an equimolar amount to 10 mg l^{-1} of Na DBS

Concentration of DBS/mg l^{-1}	Concentration of nitrate-nitrogen/mg l^{-1} Taken	Concentration of nitrate-nitrogen/mg l^{-1} Found	Error, %
2	0.1	0.112	22
5	0.1	0.149	49
10	0.1	0.220	120
2	1.0	1.06	6
5	1.0	1.12	12
10	1.0	1.26	26
2	10	10.5	5
5	10	11.1	11
10	10	12.3	23

Some studies have previously been made on the response behaviour of liquid membrane nitrate ion-selective electrodes in the presence of cationic surfactants. Takehara *et al.*[7] stated that no effect on the electrode potential was observed with the addition of 1000 mg l^{-1} of laurylamine acetate. Hulanicki *et al.*[9] stated that cationic and non-ionic surfactants exhibited an effect of minor importance at higher concentrations or even showed no effect. Small positive interferences were observed with cetyltrimethylammonium *p*-toluenesulphonate by Campi *et al.*[10] In this instance, the interference may be due to the presence of a relatively large organic anion, *p*-toluenesulphonate.

Such an interfering effect of some cationic surfactants seems to deserve further investigation. Jyo *et al.*[11] reported on the influence of a co-ion (cation) on the potential of nitrobenzene-based liquid membrane nitrate ion-selective electrode. They constructed a calibration graph using tetramethylammonium nitrate and obtained a positively curved graph at nitrate concentrations above 10^{-2} M, when the concentration of the ion pair in a liquid membrane was as low as 10^{-4} M. Their results were similar to our observations. They ascribed this interfering effect of the tetramethylammonium ion to the high extractability of the tetramethylammonium ion in comparison with the sodium ion and interpreted the results theoretically.

The interfering effect of some cationic surfactants may be interpreted by taking their extractability into account. The sequence of the effect seems to correspond to the logarithmic extraction constants given by Goto *et al.*[8] and measured in a ferron - cationic surfactant system with dichloromethane as a solvent, *viz.*, cetylbenzyldimethylammonium (5.6) >cetylpyridinium (5.2) ≈ tetradecylbenzyldimethylammonium (5.2) > cetyltrimethylammonium (5.1) > tetradecyltrimethylammonium (5.0) > stearyltrimethylammonium (4.9) > dodecyltrimethylammonium (4.6) > decyltrimethylammonium (3.6). In conclusion, the higher the extractability of cation became, the more significant was the effect of the so-called "co-ion," because the nitrate ion was the only extractable anion with cationic surfactants in solution when its concentration was sufficiently high.

Recovery Test

A recovery test was carried out with stream waters containing none and a significant amount of anionic surfactants and the results are shown in Table 3. In these two instances, a recovery of nearly 100% with a relative standard deviation of about 1% for five successive measurements were observed.

Table 2. Suppression of interference from DBS in the determination of nitrate-nitrogen using cationic surfactants. Amount of NO_3-N determined: 1 mg l^{-1}. Amount of DBS: 10 mg l^{-1}. Concentration of cationic surfactant equimolar to 10 mg l^{-1} of Na DBS

Cationic surfactant	Mean potential difference ± s.d. after 1 min/mV	Logarithmic association constant with ferron*
None	53 ± 13 ($n = 4$)	—
Decyltrimethylammonium	40 ± 6 ($n = 4$)	2.0
Dodecyltrimethylammonium	19 ± 2 ($n = 3$)	2.6
Tetradecyltrimethylammonium	6.2 + 0.3 ($n = 3$)	3.4
Cetyltrimethylammonium	4.4 ± 0.3 ($n = 10$)	3.9
Stearyltrimethylammonium	2.0 ± 0.3 ($n = 3$)	4.3
Cetylpyridinium	3.9 ± 0.2 ($n = 3$)	3.9
Tetradecylbenzyldimethylammonium	4.4 ± 0.3 ($n = 3$)	3.5
Cetylbenzyldimethylammonium	3.5 ±0.6 ($n = 3$)	3.8

* Data from reference 8.

Table 3. Recovery test with stream water

	Concentration of nitrate-nitrogen/mg l^{-1}				
Stream	Added	Found*	S.d.	Recovery, %	MBAS/mg l^{-1}
Senjyo† . .	—	0.54	0.005		0.1>
	1.0	1.54	0.025	100	
Sanda† . .	—	0.824	0.008		1.3
	1.07	1.95	0.019	103	
	2.14	3.07	0.039	104	

* Mean values of five successive measurements.

† Calibration graphs were constructed without (Senjyo) and with (Sanda) CTMA, which was added in an equimolar amount to 10 mg l^{-1} of Na DBS.

Conclusion

The method is simple and convenient for the determination of nitrate at concentrations above 0.1 mg l^{-1} in stream waters. The interference of DBS below 1 mg l^{-1} was found to be of no importance. It is possible to suppress effectively, although not completely, the interference of DBS in the concentration range 1–10 mg l^{-1} by the addition of an amount of equimolar CTMA to 10 mg l^{-1} of Na DBS. Some cationic surfactants caused the anomalous response, especially at high concentrations of nitrate. This behaviour may be interpreted by assuming that extraction of the ion pair of nitrate and the cationic surfactant occurs at higher concentrations of nitrate.

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Novel Ion-selective Electrode System for the Simultaneous Determination of Fluoride and Calcium in Acid Solution

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A method is described that utilises two independent electrode systems; a new ultra-sensitive fluoride - glass pH electrode differential cell and a calcium electrode monitor for the direct and simultaneous measurement of both fluoride and calcium in unbuffered acid solution. Fluoride Nernstian response may be extended by at least one decade towards the lower limit of detection, with an appreciable reduction in electrode response time at low concentrations when compared with the conventional use of fluoride electrodes using TISAB-type buffers. The system has been successfully applied as a biopsy technique for the determination of fluoride concentration profiles within the outer 100 μm of human tooth enamel.

Keywords: *Fluoride determination; calcium determination; acid media; ion-selective electrodes; tooth enamel*

The role of fluoride in the reduction of dental caries is not fully understood and considerable research has been directed towards the evaluation and distribution of fluoride in dental enamel, associated tissues and integuments. One current technique used for the determination of fluoride to calcium ratios in mineralised tissues depends upon the serial acid etching of small isolated areas, for example on a tooth enamel surface, followed by the analysis of individual etch solutions for fluoride and calcium using fluoride ion-selective electrodes[1] and atomic-absorption spectrometry. The calcium assay is accepted as a measure of the mass of dissolved matrix and depth of etch.[2] In a patent described by Diggens and Ross,[3] the electrochemical analysis of fluoride may be conducted at pH values below a p*K* of 3.2 for hydrofluoric acid. Under these conditions HF will be the predominant fluoride-containing species. A combination glass pH electrode and a single fluoride electrode formed a differential cell, the potential difference between the electrodes being a logarithmic function of the total fluoride concentration.

Fluoride species present with changing pH were defined as follows:

$$\mathrm{HF} \rightleftharpoons \mathrm{H^+} + \mathrm{F^-} \text{ and } K = \frac{[\mathrm{H^+}][\mathrm{F^-}]}{[\mathrm{HF}]} \quad \ldots \quad (1)$$

Response of fluoride electrode:

$$E_{\mathrm{F}} = E^{\circ}{}_{\mathrm{F}} - k\ln[\mathrm{F^-}] \quad \ldots \ldots \ldots \quad (2)$$

Response of pH electrode:

$$E_{\mathrm{H}} = E^{\circ}{}_{\mathrm{H}} + k\ln[\mathrm{H^+}] \quad \ldots \ldots \ldots \quad (3)$$

Substituting for $[\mathrm{F^-}]$ in equation (2):

$$E_{\mathrm{F}} = E^{\circ}{}_{\mathrm{F}} - k\ln\frac{K[\mathrm{HF}]}{[\mathrm{H^+}]}$$

$$= E^{\circ}{}_{\mathrm{F}} - k\ln K - k\ln[\mathrm{HF}] + k\ln[\mathrm{H^+}] \quad \ldots \ldots \quad (4)$$

Subtracting equation (3) from (4):

$$E_{\mathrm{F}} - E_{\mathrm{H}} = E_{\mathrm{TF}} = E^{\circ}{}_{\mathrm{TF}} - k\ln[\mathrm{HF}] \quad \ldots \ldots \quad (5)$$

where *E* is the observed e.m.f.; E° the standard potential; and $k = RT/zF$, the Nernstian slope factor, *R* being the gas constant, *T* the absolute temperature, *F* Faradays constant and *z* the ionic charge, including the sign. The behaviour of the combination glass pH - fluoride system conforms to a full Nernstian response, where the potential of the cell (E_{TF}) is proportional to ln[HF] or total fluoride (TF) in strong acid conditions. The patent applies to other systems of weak acids; given an electrode sensitive to small concentrations of weak acid anions and acidification below the p*K* of the protonated species, the total anion concentration may be determined using a combination pH - anion-sensitive electrode cell.

Although atomic-absorption spectrometry is the favoured technique for calcium determination, it may be inconvenient and disadvantageous compared with a non-destructive electrochemical method with the possibility of using other electrode sensors, without loss of sample. Therefore, this study describes a development of a multi-electrode system for the direct and simultaneous determination of fluoride and calcium in acid solution with a specific application to apatitic biological tissues.

Experimental

Apparatus

Two specific ion meters were used: an Orion Model 901 microprocessor digital Ionalyzer (Orion Research Incorporated, Cambridge, MA) for the direct reading of total fluoride concentration and an Orion 401 meter in the divalent cation mode for the direct readout of calcium. Both the fluoride electrode and the pH glass electrode have an extremely high electrical resistance. In order to measure the potential difference between these two electrodes, it is necessary to use an instrument such as the Orion 901, which has two high impedance inputs. In the 901, the potential of the fluoride electrode and the pH electrode are measured relative to the Ag - AgCl reference electrode, which in this instance is built into the combination pH electrode. Two separate Orion electrode systems were applied; a single fluoride electrode (94–09) in conjunction with a flat-surfaced gel-filled pH combination electrode (91–35) for fluoride determinations and a calcium electrode (93–20) together with a single-junction Ag - AgCl, 4 M KCl reference electrode (90–01). Polypropylene calibrated flasks, beakers, micropipette tips and a shallow microtitration cell (35 × 35 × 8 mm) machined from a 45 mm diameter, 20 mm thick disc of polypropylene, were employed throughout the experiments. A small PTFE-coated magnetic stirring bar (8 × 4 × 4 mm), located in a 2 mm deep recess within the centre of the titration cell, permitted vigorous agitation of 2-ml aliquots of HCl containing cumulative additions of fluoride and calcium ions. It was essential to immerse the electrode membranes completely into the test solutions and use a magnetic stirrer with an isolated electric motor to avoid changes in Nernstian response due to temperature variation.

To test the efficacy of the calibrated fluoride and calcium electrode systems in acid media, a millimetre diameter exposed surface of crystal fluorapatite was immersed for 5-min periods in the acid titration cell and the fluoride and calcium dissolution products simultaneously monitored electrochemically. Fluorapatite, of known stoicheiometric composition, was an ideal matrix with which to test the multi-electrode system, before application to apatitic biological specimens.

Reagents

De-ionised, distilled water was used throughout.

Hydrochloric acid. Dilutions were prepared from Aristar grade concentrated acid from BDH Chemicals.

Fluoride solution, 4 μg ml^{-1} *in* 0.1 M *hydrochloric acid.* Prepared from analytical-reagent grade sodium fluoride.

Calcium solution, 1 mg ml^{-1} *in* 0.1 M *hydrochloric acid.* Prepared from analytical-reagent grade calcium carbonate or hydroxyapatite.

Mineral fluorapatite, Durango, Mexico, $Ca_5(PO_4)_3F$. Calcium 39.1% (theoretical 39.7%).

Synthetic hydroxyapatite, $Ca_5(PO_4)_3OH$. Calcium 38.4% (theoretical 39.9%).

Electrode calibration solution

Fluoride. A concentration range within 0.001 to 0.1 μg ml^{-1} was obtained by the cumulative addition of 1 to 50 μl of standard fluoride solution to 2-ml aliquots of standard HCl.

Calcium. Cumulative additions of microlitre aliquots of standard or dilute standard calcium solutions to 2 ml of 10^{-1} M HCl provided a concentration range within 0.025 to 250 μg ml^{-1}. Synthetic hydroxyapatite of known composition was used as a standard for calcium electrode calibration for the analysis of fluorapatite or tooth enamel; hydroxyapatite is an appropriate standard in relation to the apatitic composition of both fluorapatite and inorganic tooth matrix.

Results

The response of the fluoride electrode in combination with a glass pH electrode in aqueous solutions of varying pH and fluoride concentration is shown in Fig. 1, which demonstrates the Nernstian response of the system. Note that the results become increasingly independent of pH below pH 2. Linear deviation for a fluoride concentration of less than 0.005 μg ml^{-1} may in part be due to nanogram amounts of fluoride in the de-ionised water and hydrochloric acid used in the experiments, which is most pronounced at pH 0.1. Using TISAB II at pH 5.5, Fig. 1 demonstrates a negligible response of the differential cell below the 0.1 μg ml^{-1} concentration of fluoride. The mean Nernstian slope factor for fluoride concentrations of 0.001–0.1 μg ml^{-1}, over a pH range of 1–4, was observed to be +55.8 (s.d. 1.6) mV pF^{-1} at 20 °C ($n = 4$), with an average correlation coefficient $r = 0.999$ (where r is the correlation coefficient calculated from regression coefficients).

At a specific pH value of 1.0 the electrode slope was observed to be +53.4 (s.d. 0.56) mV pF^{-1} at 20 °C, average $r = 0.999$, ($n = 6$) for the fluoride concentration range as above.

The time response of the differential cell is shown in Fig. 2. The mV recorder reading showed a 95% response within 30 s for a 0.005 μg fluoride addition to a vigorously stirred 1-ml sample of 0.1 M HCl, complete equilibration being attained within 2 min.

The Orion calcium electrode, a neutral carrier PVC sensor and a single-junction reference electrode containing 4 M KCl operating at an acid pH of 1.0, provided a convenient cell for the direct monitoring of calcium ion concentrations released by acid etching of mineralised tissues. The constant background ionic strength (10^{-1} M HCl) that was maintained throughout all experiments ensured a constant calcium activity coefficient and therefore calcium activity was directly proportional to concentration. Calcium calibration under the above specific conditions showed a Nernstian response over a range of 0.025–250 μg ml^{-1} of calcium, using hydroxyapatite as a standard: average electrode, slope +26.6 (s.d. 0.50) mV pCa^{-1} at 19 °C, average $r = 0.999$ ($n = 9$). Although the calcium electrode was being used at a low pH, no problems with respect to instability, drift or response were identified over a two-year period of constant use. Using the same calcium electrode module and a calcium carbonate standard following recommended direct measurement procedures at pH 6, slightly greater electrode slopes were obtained: average slope, +27.9 (s.d. 0.41) mV pCa^{-1} at 19 °C, average $r = 0.999$ ($n = 4$). A Student t-test showed a significant difference between electrode calibration using an acid solution of hydroxyapatite and calcium carbonate at pH 6 ($p < 0.001$).

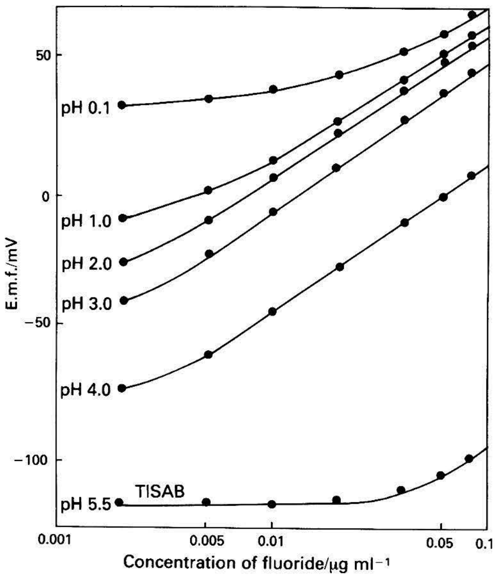

Fig. 1. Uncorrected millivolt response of the fluoride differential cell with variation of acid concentration (HCl). Extrapolation of calibration lines computed a blank value of 0.09 μg ml^{-1} of fluoride for Aristar, concentrated hydrochloric acid and 0.001 μg ml^{-1} for distilled water

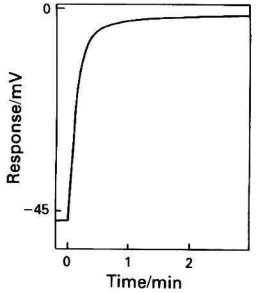

Fig. 2. Response time of the fluoride - glass pH differential cell. Using a chart speed of 20 mm min^{-1}, a 98% mV response was observed within 60 s for a 5-ng addition of fluoride to a 1-ml aliquot of 10^{-1} M HCl

As described in this paper, the application of the fluoride differential cell and the calcium electrode system to a titration cell containing 2-ml aliquots of acid, provided a technique for the simultaneous monitoring of fluoride and calcium ions. The intermittent introduction of a small window of crystal fluorapatite for serial acid etching into the titration cell, produced fluoride and calcium analyses as shown in Fig. 3.

An average fluoride to calcium ratio of 0.099 (s.d. 0.001) was determined for fluorapatite, $r = 0.999$ ($n = 12$), compared with the theoretical value of 0.095. The observed fluoride content was 3.86% (s.d. 0.05) as compared with 3.77%, the theoretical value. Assuming the density of fluorapatite (3.10), the calcium content and area of window exposed, the acid etch depth and mass of fluorapatite dissolved for each 5-min etch may be calculated, Fig. 3. Similarly, applying this technique to windowed 1 mm exposed tooth enamel surfaces, fluoride to calcium depth profiles may be obtained for the outer enamel.

Discussion

Investigation of the differential fluoride cell, which functions at a low pH, showed a Nernstian-type response extended by one concentration decade towards the lower limit of detection compared with the TISAB method for fluoride determination. This cell responds to total fluoride in the form of undissociated hydrogen fluoride, which is the predominant fluoride-containing species in acid solutions below the pK value of this acid. Diggens and Ross[3] recommended pH values to be adjusted to below 2.5 for fluoride analyses. However, as shown in Fig. 1, a Nernstian response may be attained for pH values between 3 and 4, but no response of this system was observed using TISAB at pH 5.5, for fluoride concentrations of less than 0.1 μg ml^{-1}. Extrapolation of the pH 1 to pH 4 fluoride calibration lines from Fig. 1 provided a calculated value of 0.09 μg ml^{-1} for the fluoride content of BDH Aristar HCl. This is a value for the reagent blank, necessary in order to correct for the slight curvature of response within the 0.001–0.005 μg ml^{-1} fluoride concentration range. However, this value does not account for a corrected electrode slope response of 39.7 mV pF^{-1} at pH 0.1 ($r = 0.993$) for a fluoride concentration range 0.01–0.1 μg ml^{-1}. At such pH values so few fluoride ions are present in solution that the fluoride electrode kinetics would be impaired. Although it is well known that hydrogen fluoride readily reacts with glass, no deterioration or loss in response of the glass membrane of the pH electrode was observed during 12 months of continuous use. The fast response of the fluoride differential cell and the low concentrations of fluoride analysed are not conducive to fluoride - glass reactions. The Orion calcium sensor adequately functioned at pH 1 with an unexpected life span in excess of 24 months. Adverse effects produced by calcium activity, junction potential variation or interference due to the high mobility of hydrogen ions, were reduced or avoided by using a constant background acid (ionic) strength for specimen and calibration measurements.

Although complexing ligands reduce the level of free calcium ions in solution, at low pH there will be a reduction in complexation and precipitation of insoluble calcium phosphates, carbonates, hydrogen carbonates and sulphates compared with the recommended pH 5–6 procedures for normal use of the calcium electrode. The small but significant effect of 0.1 M acid hydroxyapatite solutions on calcium electrode response was less than 5% of the slope factor when related to phosphate-free calcium solutions analysed at pH 6. With calculated correlation coefficients approaching unity for the electrode calibration, the Orion calcium electrode was shown to be quantitative over a wide range of calcium concentrations under controlled acid conditions.[4]

Serial acid etching of a fluorapatite crystal of known X-ray diffraction and chemical composition, followed by analysis of the individual etches for fluoride and calcium, confirmed the viability of the multi-electrode system. The fluoride to calcium ratio and fluoride content for fluorapatite were observed to be greater than 95% of the theoretical values. Following these evaluations, the method was successfully applied to the analysis of mineralised tissues, where the inorganic matrix is predominantly hydroxyapatite.

Electrochemical analysis of microgram amounts of surface tooth enamel as shown in Fig. 4, produced enamel fluoride profiles to a depth of 80 μm within 18 min of the analysis time. This method provides a rapid technique for the analysis of fluoride and calcium in biopsy specimens, with characterisation of the enamel fluoride profiles to a 10-μm depth within 4 min. Compared with the microlitre drop method for fluoride analysis,[5] the present extension of fluoride Nernstian response facilitates the use of larger analyte volumes, which not only reduces evaporation problems but also permits the simultaneous use of other electrode sensors without the loss of

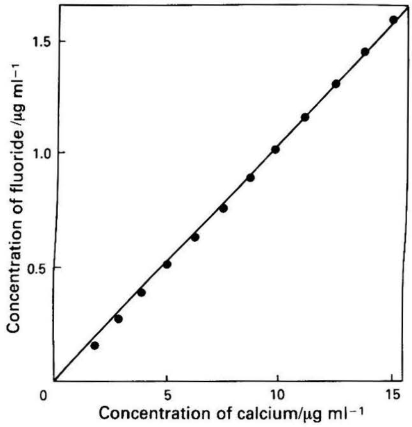

Fig. 3. Sequential analyses of mineral fluorapatite $Ca_5(PO_4)_3F$, by serial acid etching a 1 mm diameter exposed crystal surface with direct electrochemical analysis of each acid etch for fluoride and calcium. Observed fluoride to calcium ratio, calculated from regression 0.099, compared with a theoretical value for fluorapatite of 0.095. Calculated average values for the depth and mass of mineral dissolved for 5-min individual etches were 2.6 μm and 6.4 μg, respectively

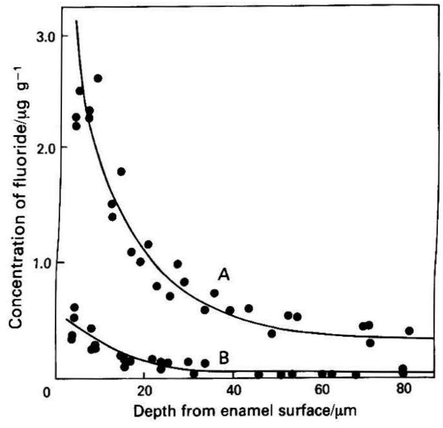

Fig. 4. Analysis of serial acid etches from 1 mm diameter enamel "windows" provided fluoride concentration profiles for a child treated with fluoride tablets since birth. A, Permanent premolar after 12 years; B, deciduous canine after 7 years. The profiles demonstrate the elevation of surface enamel fluoride, which is partially attributed to fluoride tablet therapy during permanent tooth development

fluoride sensitivity. Nevertheless, it may be possible to apply the fluoride - glass pH differential cell to a micro-drop system, in which instance nanogram amounts of mineralised tissues could be analysed for fluoride.

It is interesting to speculate as to why the fluoride electrode shows a lower limit to the Nernstian response under our experimental conditions of low pH, compared with the usually observed limit of 10^{-6} M fluoride in solution above pH 4. It is generally held that the lower limit of Nernstian response is determined by the finite solubility of the membrane used in an ion-selective electrode; within this limit, the fluoride electrode responds to fluoride released by its own dissolution. Lingane[6] suggested an enhanced solubility of lanthanum fluoride in acid media. Ikrami *et al.*[7] showed that this solubility increased with decreasing pH, and proposed a linear relationship between log (solubility) and pH. Increased solubility of the lanthanum fluoride membrane would indicate a higher, not lower limit for Nernstian response at low pH values. However, the reaction, $H^+ + F^- \rightleftharpoons HF$ leads to a reduction of fluoride ion content in solutions below pH 4. Accordingly, a solution of 10^{-6} M fluoride (at pH 5.5) would show a fluoride ion activity of only 10^{-9} M at pH 1 and we have obtained consistent, reliable results, with good recoveries of fluoride in many solutions with fluoride contents of less than 10^{-6} M.

We suggest that the lower limit of Nernstian response of the fluoride electrode is determined not only by the solubility of the membrane but also by other factors.[8] One such factor might be the reduced interference from the hydroxide ion in acid solution because, at pH 5.5 the hydroxide concentration of $10^{-8.5}$ M is significantly reduced to 10^{-13} M at pH 1. Although the hydroxide ion is a well known interferent of the fluoride electrode in low-level fluoride measurement and in alkaline solutions, the above explanation is speculative and further research will be necessary to elucidate the mechanism of fluoride electrode response at low pH.

Nevertheless, analysis of tooth enamel for fluoride and calcium contents as described in this study, yield a fluoride concentration profile related to depth of enamel. Using a constant volume of acid etchant for each profile, the stepwise cumulative observations become an analytical advantage when small fluoride and calcium increments are to be evaluated. The fast response of the fluoride differential cell together with the quantitative performance of the calcium electrode operating in an acid mode, provides a routine electrochemical technique for the simultaneous determination of fluoride and calcium in mineralised tissues.

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Evaluation of the L'vov Platform and Matrix Modification for the Determination of Aluminium in Serum

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The method previously described by Casetta *et al.* for the determination of aluminium in dilute (1 + 1) human serum using matrix modification and a stabilised temperature platform furnace has been considered. The combination of the platform, integrated absorbance, new coated tubes and oxygen addition to the charring step, provided better precision and smaller variation during the life of the tube. Good results were achieved by standardising the procedure against a calibration graph if integrated absorbance signals were used for quantitation. The calibration was linear up to at least 150 µg l^{-1} of aluminium; the within-run and between-run precision was 5.5 and 6.5%, respectively (at 14.3 µg l^{-1} of aluminium); and the recovery of aluminium added to pooled serum ranged between 97 and 102%. Furnace lifetimes in excess of 200–250 firings using oxygen ashing were routinely achieved.

Keywords: *Aluminium determination; L'vov platform; matrix modification; serum*

Several methods are used to determine the aluminium concentration in serum including neutron activation, X-ray fluorescence, flame atomic-absorption and -emission spectrophotometry, inductively coupled plasma emission spectrophotometry and graphite furnace atomic-absorption spectrophotometry.[1]

In their present state, neither X-ray fluorescence nor flame absorption/emission methods are sensitive enough to measure trace levels of aluminium in biological samples. Neutron activation analysis produces excellent results but the methods developed are time consuming and the facilities are not always readily available. Graphite furnace atomic-absorption spectrophotometry is the most frequently used technique.

Literature concerned with the determination of aluminium in a furnace is extensive,[2] but sometimes unclear. Some of the more recent developments in the atomic-absorption analysis of biological samples use various matrix modifiers,[3–5] the addition of oxygen during the ashing step to facilitate removal of carbonaceous residues,[6,7] the introduction of improved pyrolytically coated graphite tubes[8] and the stabilised temperature furnace with the L'vov platform.[9–12]

In deciding which analytical approach (wet ashing, extraction procedure, direct injection etc.) to adopt for routine use in a clinical laboratory, we considered three fundamental criteria. These were that the method should be simple, rapid and that sample pre-treatment should be minimal to reduce the source of contamination.

In this work the approach based on the direct injection of dilute (1 + 1) serum with accurate control of instrumental conditions, proposed by Casetta *et al.*,[13] has been evaluated as it appeared to meet the above criteria. In this technique dilution with a matrix modifier is necessary to reduce the viscosity of the samples; an injection volume of 10 µl is suitable to prevent improper deposition of sample on to the walls of the tube; the sampling capillary tip must be aligned perfectly (adjusting the tip distance from the platform and the tip dipping in the cup) with the sample introduction hole in the graphite tube; and the serum in the cup must be shaken well before the sample is placed on the platform; a programme step must be added between char and atomisation (at the same char temperature but at a "gas stop" condition) to minimise the temperature difference between the char and atomisation steps.

Experimental

Apparatus

A Perkin-Elmer Model 5000 atomic-absorption spectrophotometer, equipped with an HGA-500 graphite furnace, AS-40 autosampler and a Model 056 strip-chart recorder was used for the absorbance measurements. A deuterium arc lamp was used to correct for non-specific absorption. New pyrolytically coated graphite tubes with a solid pyrolytic graphite platform were used. The purge gas was argon except in the charring step where oxygen was employed.

Reagents

All of the water used to wash laboratoryware and prepare solutions and standards was prepared by a Milli-Q system (Millipore Ltd.). All glassware, plastic tubes and stoppers, disposable pipette tips, polypropylene cups for the AS-40 sampler etc., were pre-cleaned by soaking them overnight in a saturated solution (about 0.5 mol l^{-1}) of disodium ethylenediaminetetracetate (BDH Chemicals) rinsed with copious amounts of de-ionised water and dried in a clean atmosphere.

Serum samples were diluted with an equal volume of a solution containing 2.0 g l^{-1} of $Mg(NO_3)_2$ (Merck Suprapure grade); no Triton X-100 solution was added to the sample.

A stock solution of 100 mg l^{-1} of Al (aluminium nitrate, BDH standard solution for AAS) was used to prepare the standards. From this solution, a 1 mg l^{-1} Al intermediate stock standard was prepared daily; 2.5, 5.0, 10.0 and 15.0 ml of this solution were pipetted into separate polypropylene containers and made up to 100 ml with de-ionised water. These standards correspond to 25.0, 50.0, 100.0 and 150.0 µg l^{-1} of aluminium, respectively, and were used for the standard additions method.

Sample Collection

Blood samples, obtained by a plastic cannula with stainless-steel needles, were transferred into 10-ml polypropylene sample tubes, centrifuged at 2000 rev min^{-1} for 10 min and the serum was transferred into 3-ml plastic containers. In the determination of reference values, samples were taken from "normal" persons through a plastic cannula that had been washed with blood. For each sample, a double 10-ml portion of blood, was then collected, centrifuged as above and stored at 4 °C. Sera determined to have a low aluminium content by the automated method of standard additions were pooled and used as the matrix to prepare calibration graphs. All serum samples were analysed within four weeks to date of sampling and to minimise contaminations all operations were performed under dust covers.

Results and Discussion

The instrumental operating conditions for the HGA-500 (Table 1) were those reported by Casetta *et al.*[13] In this study, the linearity of the calibration graph, the sensitivity, the within-run and between-run precision, the detection limit, the analytical recovery and the tube life have been verified. Graphite furnace analyses were occasionally subject to a random error resulting from a system malfunction. To detect these, we repeated the determination on each sample at least three times. A random result clearly variant from the others was rejected and the remainder were averaged. As evident from the calibration graph (Fig. 1) linearity up to at least 150 µg l^{-1} of Al was obtained. Using 10 µl of dilute (1 + 1) serum the sensitivity of the platform system was 1.05 µg l^{-1} of Al for 0.0044 absorbance seconds. In order to study the need for background correction, 50 and 100 µg l^{-1} of Al were added to aliquots of pooled serum and the analyses were performed with and without using background correction. The difference between the absorbances were, in both the tests, within the analytical variation of the method, which indicated that background correction was unnecessary at this wavelength.

Precision data are presented in Table 2. Within-run precision was estimated by analysing a serum pool that was spiked, divided into aliquots and kept at 4 °C until absorbance measurements were made. Between-run precision was obtained from the analysis of two serum pools performed on separate days. Recovery studies were performed by adding two known amounts of aluminium to aliquots of three serum samples. The original serum and the two spiked aliquots were processed and the calculated recovery data are given in Table 3. The detection limit (2σ) measured with a 20 µg l^{-1} Al standard was 2.0 pg of Al.

In one test a serum sample (containing about 100 µg l^{-1} Al) was fired repetitively in a pyrolytically coated graphite tube until the tube failed. Figs. 2 and 3 show the chart recorder response of this serum sample after ten and 150 firings, respectively. After 150–180 firings there was no systematic change in the aluminium peak area signal, but a remarkable change in the peak height signal. A good example of the advantage of using area integration is reproduced in Fig. 4, which shows the change in peak shape recorded by increasing the firings.

Table 1. Instrument and furnace conditions for the determination of aluminium in serum: Model 5000 spectrophotometer; hollow-cathode lamp current, 20 mA; wavelength, 309.3 nm; slit width, 0.7 low; background corrector, yes; signal, peak area; integration time, 6 s; chart recorder, 20 mm min^{-1}; and span, 10 mV full scale. HGA-500 graphite furnace; pyrolytically coated graphite tubes with a stabilised temperature platform furnace; sample volume, 10 µl; alternative volume, 10 µl

	HGA programme								
Step	1	2	3	4	5	6	7	8	9
Temperature/°C	80	130	500	1500	1500	1500	2400	2600	20
Ramp/s	1	30	30	1	15	1	0	1	1
Hold/s	4	25	55	25	30	4	6	6	20
Flow-rate of internal gas Ar/ml min^{-1}	300	300		300	300	0	0	300	300
Flow-rate of alternative gas O_2/ml min^{-1}			50						
Recorder							−10		
Read							X		
Base line							X		

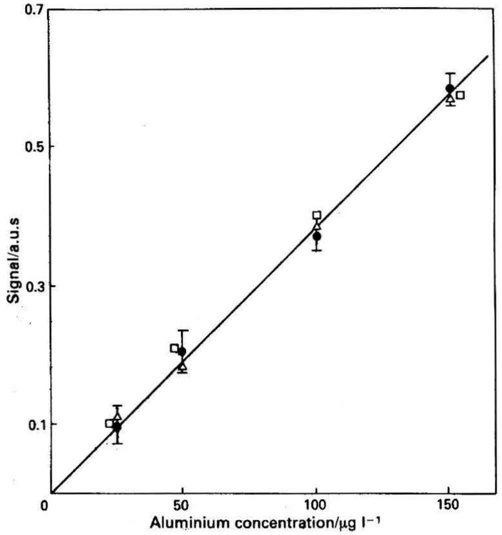

Fig. 1. Calibration graph for aluminium in dilute (1 + 1) serum using the pyrolytically coated graphite tube and the L'vov platform. Standard additions method results: △, from pooled sera at low aluminium content (five determinations); ●, from 50 serum samples analysed on different days with five different tubes. The natural level of aluminium has been subtracted from all readings. □, Results from aqueous solutions

Table 2. Precision data for the determination of aluminium

		Relative standard deviation, %	
Sample	Pool/µg l^{-1}	Within-run*	Between-run†
Serum A	14.3	5.5	6.5
	+50.0	3.6	4.1
	+100.0	1.5	2.7
Serum C	39.2	3.1	4.6
	+50.0	1.1	3.1
	+100.0	0.7	2.6

* Results of five determinations.

† Calculated on five separate days.

Table 3. Recovery data of aluminium added to pooled sera

Sample	Pool/ µg l^{-1}	Added/ µg l^{-1}	Found/ µg l^{-1}	Recovery, %
Serum A . .	4.3	50.0	55	102
		100.0	103	99
Serum B . .	17.0	50.0	66	99
		100.0	119	102
Serum C . .	39.2	50.0	86	97
		100.0	136	98

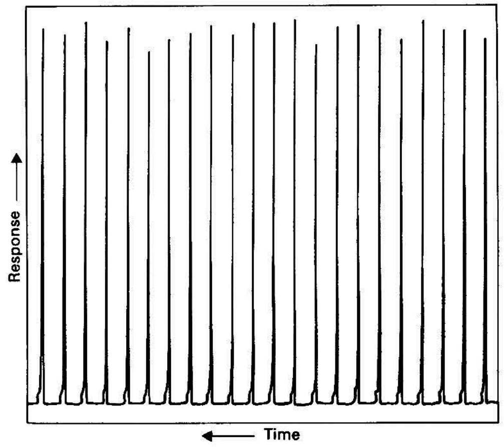

Fig. 2. Chart recorder response (10 ×) of 100 µg l^{-1} of Al in serum after ten firings. Peak area signal reproducibility (coefficient of variation) 2.20%

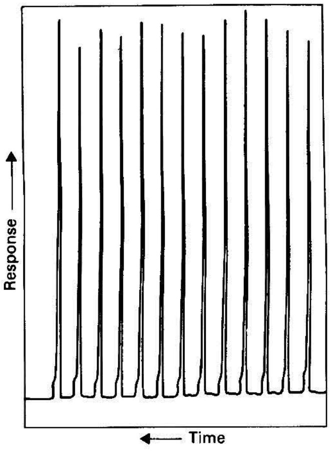

Fig. 3. Chart recorder response (10 ×) of 100 µg l^{-1} of Al in serum after about 150 firings. Peak area signal reproducibility (coefficient of variation) 2.50%

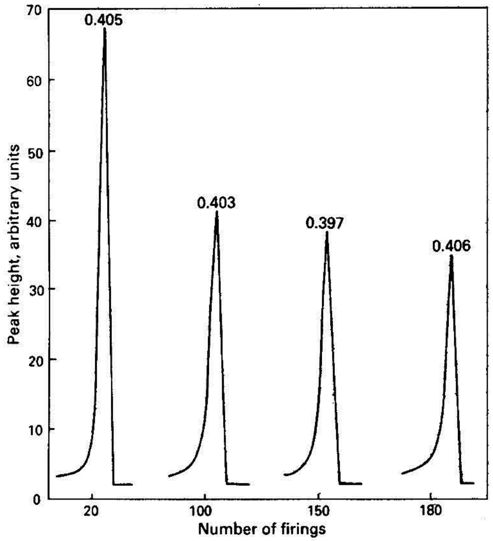

Fig. 4. Change in peak shape with increased number of firings. Signal of 1 ng of Al in serum from a platform in a pyrolytically coated tube. Values on peaks are signal areas in absorbance seconds

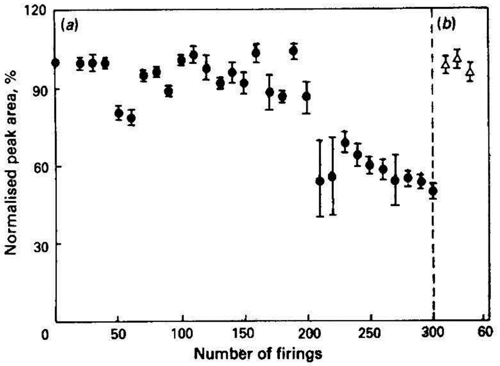

Fig. 5. Signal repeatability of a dilute (1 + 1) serum sample during the life of the pyrolytically coated graphite tube with the L'vov platform. Average of five determinations (+1σ) is plotted every ten firings. The tube failed after 400 firings. (*a*) Tube No. 1; (*b*) tube No. 2 with the "old" platform

Fig. 5 shows the repeatability of the aluminium peak area signal at intervals of ten firings. Each point of the graph represented an average of five firings. This particular tube failed after about 400 firings of diluted serum. Fig. 5 also shows the progressive loss in sensitivity of tube (1) (after about 200 firings), which might be attributed to the erosion of the pyrolytic inner surface.

Using the same "old" platform and a new pyrolytic tube, the normalised peak area signal agreed very well with the earlier sets of data. No ash build up was found after about 300 firings of dilute serum samples.

In another test we used five pyrolytically coated tubes and five graphite platforms to analyse serum samples. Any one of these tubes showed a precision, after about 150 firings, of less than 5.9% at 100 µg l^{-1} of Al. Furnace lifetimes in excess of 200–250 firings using oxygen ashing, were routinely achieved.

In this study 198 serum samples were analysed by the method of standard additions and on pooling the results a line with slope = 38.8 × 10^{-4}, *y*-intercept = 9.0 × 10^{-4} and r = 0.998 was produced. This line (Fig. 1) was not significantly different from the calibration graphs prepared initally with pooled sera with low aluminium content (slope = 38.0 × 10^{-4}; *y*-intercept = 7.5 × 10^{-4}; and r = 0.988) and with aqueous solutions (slope = 38.6 × 10^{-4}; *y*-intercept = 1.2 × 10^{-4}; and r = 0.999).

Results obtained for 169 serum samples by the method of standard additions minus those from the calibration graph did not differ significantly [mean difference = +2.05 ± 3.99 (1 s.d.) µg l^{-1} of Al]. The concentration of aluminium in sera ranged from 4 to 108 µg l^{-1} of Al. From these results it was established that for an accurate determination of aluminium in serum the method of standard additions is not necessary and a single calibration procedure utilising aqueous solutions is adequate.

The calibration method permits the direct calculation by microprocessor of the aluminium concentration from a single calibration point using the specific function available on the Model 5000 spectrophotometer. To obtain reliable results it is important, particularly as the tube ages, to check that the calibration has not changed (recalibration function). The aluminium concentration in serum from healthy subjects ($n = 40$) was determined to be 17.3 ± 6.1 (range 2.0–36.0 µg l^{-1} of Al). The main criterion used for including samples was that the donor was not taking aluminium-containing antacids. Serum aluminium concentration in 68 samples (haemodialysis patients and patients on continuous ambulatory peritoneal dialysis) was 29 ± 16 µg l^{-1} Al (range 8–106 µg l^{-1} Al). A significant statistical difference between these two groups of data was present, as the *t*-test for the mean produced a value of $p < 0.001$. Further suitable considerations such as sex and age of the subjects, type and length of treatment etc., will be presented in a later paper.

Many workers have suggested that sample contamination during blood collection can contribute to the higher aluminium values in normal subjects. In order to verify that all manipulations were performed without the introduction of random contaminations we collected and analysed, separately, two portions of serum for each subject (87 samples) and four aliquots of serum for one normal subject. The aluminium concentrations determined in the four serum specimens were respectively, 16, 17, 16 and 18 µg l^{-1} of Al (mean 16.7 ± 1.0) and the results obtained for 84 samples (16 normal subjects and 68 patients) did not differ significantly (*t*-test for paired observations produced a value of $p > 0.01$). In this test only three samples were found to give anomalous results and were rejected. (a) 64 and 35 µg l^{-1} of Al; (b) 25 and 44 µg l^{-1} of Al; and (c) 24 and 44 µg l^{-1} of Al. Samples (a) and (c) were "normal" subjects and (b) was a haemodialysis patient.

Conclusions

The results obtained show that the determination of serum aluminium concentration using the stabilised temperature furnace with chemical matrix modification is acceptable. Reliable results can be achieved by standardising the procedure against a calibration graph if integrated absorbance signals are used for quantitation. The combination of the platform, matrix modification, integrated absorbance, oxygen addition to the charring step and new coated tubes provided better precision and smaller variation during the life of the tube.

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Determination of Electroactive and Non-electroactive Gases Using a Membrane Polarographic Detector in a Flow System

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A simple method has been devised for analysing toxic corrosive gases, such as Cl_2, NO_2 and SO_2, using an oxygen membrane polarographic detector (O_2-MPD) coupled to a flow system, and an inert carrier gas, such as N_2. The flow system minimises the degree of corrosion and poisoning of the MPD so often found when analysing these gases. It was shown that, by using an electroactive carrier gas such as air (instead of N_2), the same O_2-MPD - flow system could also be used to detect non-electroactive gases, such as N_2, Ar, He, CH_4 and CO_2. The peak current observed, following injection of an electroactive, or non-electroactive gas, was found in all instances to be directly related to the amount injected. The application of MPD's in gas chromatography is discussed in the light of this work.

Keywords: *Electroactive gas determination; non-electroactive gas determination; flow system; membrane electrode; Clark cell*

Following the development of the oxygen membrane polarographic detector (O_2-MPD) by Clark,[1] many groups[2–10] have tried, with varying degrees of success, to develop MPD's for gases other than O_2. Indeed, quite early on, Sawyer *et al.*[2] showed that Cl_2, Br_2, NO_2 and SO_2 could be detected using an O_2-MPD, whereas H_2S, H_2 and N_2 could not. Over the last decade a great deal of progress has been made in this area. For example, Ben-Yaakov,[3] using a porous membrane, constructed an MPD for the determination of Br_2, Cl_2, I_2 and the acids HOCl and HOBr; Albery and co-workers developed MPD's for N_2O[4] and CO_2[5], and more recently we developed ones for H_2[6] and D_2.[7] In addition to these developments with conventional "Clark-type" MPD's, Bergman and co-workers[8–10] have shown that the "metallised membrane electrode" (which does away with the electrolyte layer and all the complications it affords) can be used to detect O_2, H_2, CO and NO_2. Subsequent work by Pletcher and Gibbs[11] confirmed these findings, and showed that this type of electrode could be also used to detect SO_2 and NO. A detailed report by Bergman on the polarographic curves for many oxidising and reducing toxic gases (such as Cl_2, NO_2, SO_2, NO and H_2S), recorded using a metallised membrane electrode, has been published recently.[12] However, as has been noted by both Sawyer *et al.*[2] and Bergman,[12] detection of large amounts (*i.e.*, partial pressures *ca.* > 0.5 atm) of some of these toxic gases can prove difficult. For example, both these workers noted some degree of corrosion of their MPD's whilst monitoring high levels of Cl_2, NO_2 or SO_2. Thus, high partial pressures of these gases can lead to the permanent damage of an MPD as well as erratic and irreproducible readings. Indeed, to avoid problems of this nature, Bergman used only dilute forms of these gases (usually 1%) in his polarographic study.[12]

In addition to corrosion of the MPD, erratic and irreproducible results can also occur if the working electrode is poisoned by the electroactive gas. Sawyer has reported[2] this to be so for SO_2 and H_2S, with the latter gas appearing particularly effective as a poison. Pletcher *et al.*,[13] using a metallised membrane electrode, have reported that CO will also poison the working electrode (in this instance, Au or Pt supported on a PTFE membrane) of an MPD. As with the problem of corrosion, dilution of the electroactive gas with an inert gas (such as N_2) appears to offer a partial solution to the problem, as it reduces the rate of deactivation of the electrode surface.

In the first part of this paper we describe how a variety of toxic electroactive gases can be detected, and how the problems of poisoning and corrosion can be reduced substantially by using an O_2-MPD coupled to a flow system, with an inert carrier gas (such as N_2).

The second part of the paper is concerned with what, at first sight, appears to be a contradiction in terms, *i.e.*, the detection of non-electroactive gases using an O_2-MPD. In it we describe how this rather unusual situation can be achieved by using the same O_2-MPD coupled to a flow system as mentioned in the previous paragraph, but, this time, with an electroactive carrier gas (such as air).

Experimental

Apparatus and Reagents

The main components of the flow system used are shown in Fig. 1. A fine control of carrier gas flow-rate was achieved using an Edwards needle valve (Model LB1 B) attached at the head of the cylinder (1). Flow-rates were measured using a calibrated flow meter (2) (Glass Precision Engineering Ltd., Model RS1/C) incorporated in the line (see Fig. 1). The carrier gas flowed from the cylinder (1) into the mixing chamber (3) [a 125-cm^3 Pyrex Dreschel bottle, modified to receive a rubber septum (4)] and then on to the O_2-MPD (5). It is worth noting that as the O_2-MPD measures the partial pressure of the electroactive gas it is important to keep the flow-rate (and, therefore, the pressure of the gas reaching the detector) stable. A detailed description of the O_2-MPD (5) (designed and constructed in this department, and now available commercially from Rank Bros., Cambridge) has been given in a previous paper.[14] The gases Cl_2, NO_2 and SO_2 were supplied by BDH Chemicals Ltd., all others were obtained from BOC.

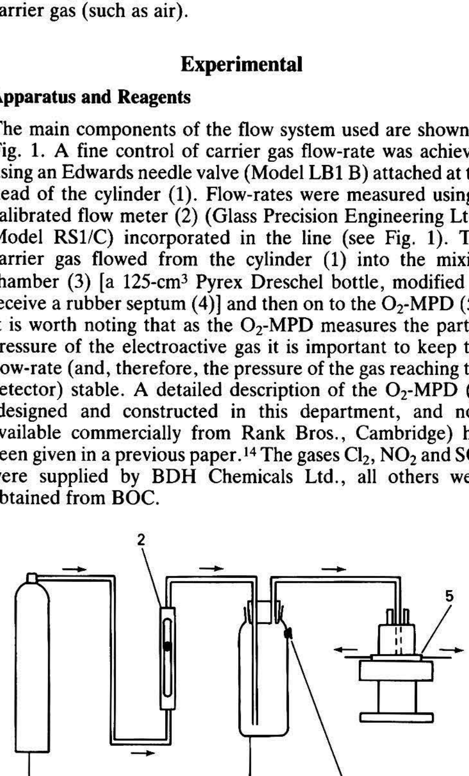

Fig. 1. Schematic diagram of the flow system coupled to the O_2-MPD. The components are: 1, carrier gas cylinder; 2, flow meter; 3, mixing chamber; 4, rubber septum, and 5, O_2-MPD

Procedure

Nitrogen (white spot, BOC) was used as the carrier gas in the work on electroactive gases, whereas air (BOC) was used in the work on non-electroactive gases. In either instance, small volumes (usually $0.1 \leq V_i \leq 30$ ml) of the gas under study (electroactive, or non-electroactive) were injected, via the rubber septum (4) (see Fig. 1), into the mixing chamber (3), and then swept out, by the appropriate carrier gas, to the O_2-MPD for detection. In all of this work the Pt working electrode of the O_2-MPD was polarised at −0.7 V *versus* the Ag - AgCl counter - reference electrode, using either a Metrohm potentiostat (Model E611) or a potentiostat designed by Mills and Pavlou and now available commercially from Rank Bros. (Cambridge). Both potentiostats had a compensation current facility, which was essential for the work on non-electroactive gases. The output of the O_2-MPD was recorded on a Servogore 210 *x* - *t* chart recorder.

Results and Discussion

Detection of Toxic Electroactive Gases

The electroactive gases selected for study (*i.e.*, Cl_2, NO_2 and SO_2) are good oxidising agents and, therefore, will not only be readily reduced at the working electrode of an O_2-MPD but will also corrode and damage other parts of it (*e.g.*, the silicone rubber retaining ring[2]). In addition, as discussed in the introduction, SO_2 can poison the surface of the working electrode (particularly Pt) of an O_2-MPD.[2] The extent of corrosion and/or poisoning of an O_2-MPD by any one of these electroactive gases will, amongst other things, depend upon: (*a*) the partial pressure of the gas and (*b*) the duration of its exposure to the detector. Thus, when using an MPD to detect such gases, it is important to minimise both (*a*) and (*b*), and this we can do by coupling the detector to a flow system, see Fig. 1. The partial pressure of the electroactive gas (P_{eg}) immediately after ($t = 0$) injection into the mixing chamber, *assuming perfect mixing*, would be given by

$$P_{eg} = \frac{V_i P'}{V_o} \quad \ldots \ldots \ldots \ldots \quad (1)$$

where V_i = volume of electroactive gas injected (cm³); V_o = volume of the mixing chamber (cm³); and P' = the total pressure in the mixing chamber (atm).

This would then be expected to decay exponentially as the electroactive gas is swept out of the mixing chamber by the inert carrier gas to the MPD.[14] The subsequent response of the MPD would be given by

$$i_d(t) = \frac{V_i}{V_o} i_{eg} \exp(-ft/V_o) \quad \ldots \ldots \quad (2)$$

where $i_d\ (t)$ = diffusion-controlled current at time t after injection (μA); i_{eg} = diffusion-controlled current when the electroactive gas, instead of N_2, is flowed through the system (μA); and f = flow-rate of the carrier gas (cm³ min⁻¹).

Equations (1) and (2) have been verified recently using O_2 as the electroactive gas.[14] They imply that the degree of poisoning and/or corrosion of an O_2-MPD, by a toxic electroactive gas, such as Cl_2 or SO_2, can be minimised by incorporating the MPD into a flow system using: high flow-rates (f), low injection volumes (V_i), and a large volume mixing chamber (V_o). This is because, under these conditions, the partial pressure and exposure time of the electroactive gas to the O_2-MPD would be relatively small.

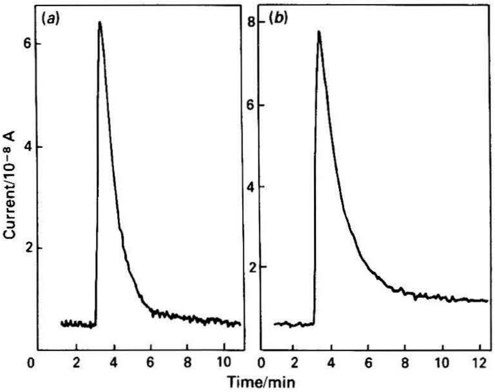

Fig. 2. Typical output from the O_2-MPD following an injection of 5 ml of (*a*) NO_2, and (*b*) SO_2 (third injection) into the mixing chamber of the flow system, using N_2 as the carrier gas (f = 310 cm³ min⁻¹). The time origin is arbitrary

Table 1. Data on non-electroactive gases

Property	Air	NO_2	Cl_2	SO_2
Least-squares analysis*				
No. of points . .	8	7	8	7
Gradient/nA ml⁻¹	13.7 ± 0.1	12.2 ± 0.1	47.5 ± 0.4	14.3 ± 0.4
Correlation coefficient . .	0.9998	0.9999	0.9998	0.9986
Peak half-life ($t_{½}$)†/s . .	20	30	30	36‡
Permeability in PTFE at NTP §/ . .	0.87	1.39	—	1.25

* For a plot of $i_p{}^0$ *versus* V_i over the range $0.2 \leq V_i \leq 5$ ml; f = 310 cm³ min⁻¹.

† $t_{½}$ = time taken for $i_d(t) = i_p{}^0/2$.

‡ This is $t_{½}$ for the first injection, $t_{½}$ increases thereafter with each subsequent injection [see Fig. 2(*b*)].

§ From reference 11; units, 10^{-7} cm⁻³ per cm atm s.

A large variety of volumes (V_i = 0.2–5 ml) of each electroactive gas (*i.e.*, air, NO_2, Cl_2 and SO_2) were injected into the mixing chamber of the flow system (see Fig. 1). In order to minimise the contact time between the O_2-MPD and the gas under study, a high flow-rate (f = 310 cm³ min⁻¹) was employed. Fig. 2(*a*) shows a typical current *versus* time output from the O_2-MPD on injection of 5 ml of one of these electroactive gases (in this instance, NO_2). Ideally, the peak current ($i_p{}^0$), should be given by

$$i_p{}^0 = \frac{V_i}{V_o} i_{eg} \quad \ldots \ldots \ldots \ldots \quad (3)$$

i.e., $i_p{}^0$ should be directly related to the volume of the electroactive gas injected (V_i). This was, in fact, found to be so, and the results of a simple least-squares analysis of the data for an $i_p{}^0$ *versus* V_i plot for each gas, are given in Table 1. From Table 1 we can see that the flow system provides an excellent method for the quantitative analysis of gaseous samples containing large amounts of an electroactive component, such as O_2, Cl_2 and NO_2, and in all this work none of the problems[2] due to MPD corrosion were encountered. However, although corrosion of the MPD appeared minimal, some poisoning of the Pt working electrode by SO_2 was observed, particularly when large (V_i > 3–5 ml) injections were made. Unlike the other electroactive gases used, large injections of SO_2, led to current *versus* *t* profiles that changed in shape after each injection. Fig. 2(*b*) shows a typical output from the O_2-MPD after a third injection of 5 ml of SO_2; the first injection appearing very similar to that of NO_2 [see Fig. 2(*a*)]. The poisoning of the Pt working electrode by SO_2 appeared to manifest itself in the form of peak broadening, and an increase in the residual, or base line, current after each injection, however, the peak current ($i_p{}^0$) (as measured with reference to the pre-injection base line) appeared less affected. The effects of poisoning decreased with decreasing volume of SO_2 injected, becoming unnoticeable when V_i < 1 ml.

In a previous paper[14] we showed that the time taken for $i_d(t)$ to reach $i_p^0/2$, *i.e.*, the half-life of a peak ($t_{\frac{1}{2}}$), should be

$$t_{\frac{1}{2}} = \frac{V_o}{f} \ln 2 \quad .. \quad .. \quad .. \quad .. \quad (4)$$

for an "ideal" MPD, *i.e.*, one with a zero response time. As in all our work V_o = 156 ml, and in this study f = 310 ml min^{-1}, then using equation (4) we can calculate that $t_{\frac{1}{2}}$ = 21 s. In Table 1 are listed typical half-life values observed for a 5-ml injection of each gas. In agreement with our previous work,[14] equation (4) appears to hold when O_2 is the electroactive gas. However, a slightly more sluggish response by the O_2-MPD was observed for Cl_2, SO_2 and NO_2, and is probably due to the interaction of these gases with the electrolyte layer to form acids (*e.g.*, HOCl, H_2SO_3 and HNO_2) which can subsequently diffuse to, and then react at, the Pt working electrode. This effect could be avoided if a metallised membrane electrode, rather than a Clark-type electrode, is used, and this indicates an important advantage of the Bergman-type electrode.[12]

The gradients from the i_p^0 *versus* V_i plot for each gas, given in Table 1, correspond to the average peak currents per ml of gas injected. The peak current is determined by, amongst other things, (*a*) the degree of mixing with the carrier gas, (*b*) the permeability of the electroactive gas toward the membrane material (in this instance, PTFE) covering the sensing electrodes and (*c*) the degree of catalytic activity shown by the Pt working electrode towards the reduction of the gas. Similar peak shapes and $t_{\frac{1}{2}}$ values indicate that, at these high flow-rates (f = 310 cm^3 min^{-1}), the degree of mixing of the electroactive gas with the carrier gas is similar for all gases studied. As the permeabilities of these gases towards PTFE are also similar (see bottom of Table 1), it appears likely that the large differences in peak current between O_2 and Cl_2 on the one hand, and NO_2 and SO_2 on the other, are due to the catalytic factor outlined in (*c*).

So far we have concerned ourselves with only the detection of toxic oxidising gases, however, it is obvious that any gas that can be made to react at the working electrode of an MPD (these include: O_2, Cl_2, Br_2, I_2, NO_2, NO, N_2O, CO, CO_2, SO_2, H_2S, H_2 and D_2) could be analysed using a flow system coupled to an MPD. As mixtures of such gases could be separated by chromatography, they too could be analysed using such a system. However, by its very nature, the MPD is limited to the detection of electroactive gases, and in the next section we describe how this major problem can be overcome simply, with the result that non-electroactive gases can be detected using the same O_2-MPD coupled to a flow system as described here.

Detection of Non-electroactive Gases

If air, instead of N_2, is used as the carrier gas in the flow system (see Fig. 1), then it should be possible to "detect" non-electroactive gases using the O_2-MPD, as such gases will cause a transitory lowering of the partial pressure of O_2 in the mixing chamber on injection, which can be subsequently monitored, down the line, by the O_2-MPD. Assuming perfect mixing, the current *versus* time output of the O_2-MPD following injection of V_i cm^3 of a non-electroactive gas into the mixing chamber would be

$$i_d(t) = \frac{i_{air}}{V_o}[V_o - V_i \exp(-ft/V_o)] \quad .. \quad .. \quad (5)$$

where i_{air} = diffusion-controlled current when only air is flowing through the system.

It follows from equation (5), that the maximum drop in current, which we shall call the peak current (i_p'), and which should be observed immediately (t = 0) following injection, can be written as

$$i_p' = \frac{V_i}{V_o} i_{air} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

Fig. 3(*a*) shows a typical current *versus* time output from the O_2-MPD immediately following injection of 5 ml of N_2 into the flow system (f = 180 cm^3 min^{-1}). The observed peak current of 64 nA is in good agreement with that predicted by equation (6), where i_p' = 70 nA (as V_i = 5 ml, V_o = 156 ml and i_{air} = 2.2 μA). In addition, as predicted by equation (5), the decay of the peak current, i_p', is exponential, and a plot of $\ln[i_d(t) - i_{air}]$ *versus t* yields a reasonable straight line (r = 0.9976) over two half-lives, with a gradient (m) = 1.21 ± 0.04 min^{-1}. This value for the gradient agrees well with that predicted by equation (5), *i.e.*, m = 1.15 (= f/V_o). However, injection of other non-electroactive gases (*i.e.*, gases that cannot be reduced at the Pt working electrode of the O_2-MPD), such as He, Ar, CH_4 and CO_2, did not lead to similar "ideal" responses from the O_2-MPD. From Table 2 we can see that the typical peak currents (i_p'), recorded for a 5-ml injection of each gas, are dissimilar. Leaving aside CH_4 and CO_2, it is interesting to note that the peak currents are in the order He > N_2 > Ar, although the peak areas are approximately (±5%) equal. As the densities of these three gases are in the order Ar > N_2 > He, it may be that the trend in i_p' reflects different degrees of mixing of the injected inert gases with the carrier gas, possibly due to the low flow-rate (f = 180 cm^3 min^{-1}). For CH_4, however, the peak current is greater

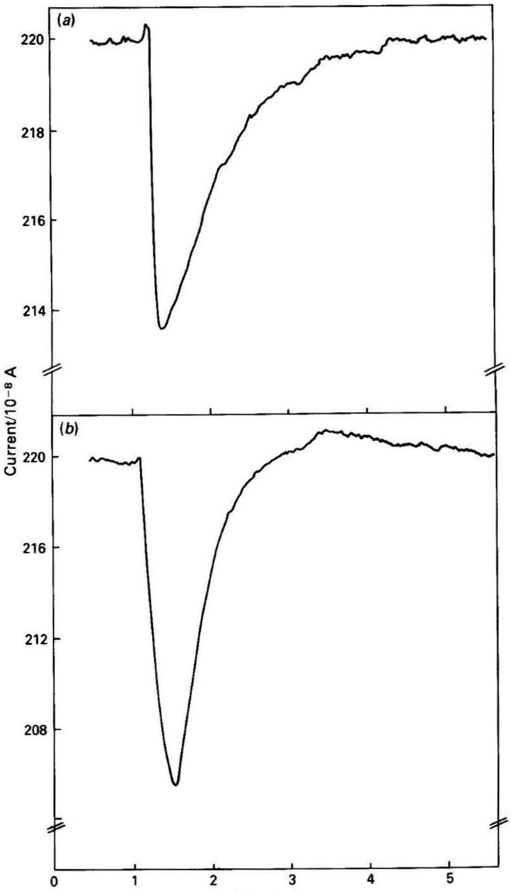

Fig. 3. Typical output from the O_2-MPD following an injection of 5 ml of (*a*) N_2 and (*b*) CO_2 into the mixing chamber of the flow system, using air as the carrier gas (f = 180 cm^3 min^{-1}). The time origin is arbitrary

Table 2. Data on non-electroactive gases

Property	N_2	Ar	He	CH_4	CO_2
Typical peak current*/nA	64	47	78	86	154
Typical peak area/nA min	67	61	64	58	—
Result of least squares analysis†					
No. of points	12	12	—	16	13
Correlation coefficient	0.9993	0.9972	—	0.9988	0.9960

* For a 5-ml injection of gas; f = 180 cm^3 min^{-1}.

† For a plot of i_p' *versus* V_i over the range V_i = 30–0.2 ml; f = 260 cm^3 min^{-1}.

than that of He even though its density is lower. It is probable that this is due to the adsorption of CH_4 on to the surface of the Pt working electrode, and subsequent inhibition of the reduction of O_2, and possible oxidation of the adsorbed CH_4.

All the gases discussed so far, gave similar peak shapes, *i.e.*, an initial high peak current, following injection, which then decayed exponentially, but with CO_2 this was not found to be so and Fig. 3(*b*) shows a typical response of the O_2-MPD following injection of 5 ml of CO_2 into the mixing chamber. Possible causes for this anomolous behaviour include: alteration of the pH of the electrolyte surrounding the Pt working electrode, due to carbonic acid formation, and adsorption of CO_2 on to the electrode surface. Despite this unusual peak shape, the peak current (i_p') was found to be directly related to the amount of CO_2 injected. Indeed, this was found to be so for all the gases used in this study (*i.e.*, CH_4, CO_2, N_2, He and Ar) over the range $0.2 \text{ ml} \leq V_i \leq 30 \text{ ml}, f = 260 \text{ cm}^3 \text{ min}^{-1}$. At the bottom of Table 2 are listed the correlation coefficients, along with the number of points used in its calculation, from a plot of i_p' *versus* V_i for each gas.

In conclusion, by using an electroactive carrier gas, such as air, it has proved possible to detect quantitatively non-electroactive gases (*i.e.*, gases that cannot be reduced at the Pt working electrode of the O_2-MPD) with an O_2-MPD coupled to a flow system (see Fig. 1). Quantitative analysis of a mixture of non-electroactive gases (or, electroactive gases for that matter) using an O_2-MPD would require separation (by gas chromatography, for instance) of the mixture prior to detection, due to the limited selectivity of the O_2-MPD. This idea is not original however, as Bergman *et al.*[10] have already shown that mixtures of CO and H_2 could be separated by gas chromatography and subsequently detected using a metallised membrane electrode. In addition, Blurton and Stetter[15] have used a PTFE-bonded diffusion electrode as the detector in the gas chromatography of many electroactive gases, including H_2S, NO, CO, SO_2 and NO_2. So far, the use of MPD's in gas chromatography has been limited to the detection of electroactive gases, but from the work described in the latter part of this paper we can now see that non-electroactive gases may also be detected using an MPD, after separation by gas chromatography, provided an electroactive carrier gas is used. Indeed, just recently we have shown that the major components of petroleum spirit (b.p. 40–60 °C) (including 2-methylbutane, pentane, 2,2-dimethylbutane, 2,3-dimethylbutane, 2-methylpentane, 3-methylpentane and hexane) can be detected after separation by gas chromatography, using an H_2-MPD and H_2 as the carrier gas. A detailed account of this work will appear at a later date, but the principles upon which it is based are those described in the latter part of this paper. Interestingly, the idea of indirect detection is not altogether new to chromatography; indeed, its use, in the form of indirect UV detection, is becoming increasingly popular in ion chromatography.[16]

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Preparation of Coated Plastic or Glass Rod Epoxy-based Voltammetric Electrodes Using a Multi-layer Coating and Vapour Hardening Technique

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A method is recommended for producing graphite-loaded epoxy-based voltammetric electrodes by coating plastic or glass rods. A multi-layer coating technique is used, each coating being hardened by absorbed hydrofluoric acid vapour. Electrodes of sufficiently low electrical resistance are produced by rubbing graphite into several of the lower coatings immediately after they are hardened. Unlike coated-wire electrodes complete coverage of the surface being coated is not essential.

Keywords: *Graphite-loaded epoxy-based electrodes; hydrofluoric acid vapour hardening; voltammetry; coated rod electrodes*

Previously,[1] a new multi-layer coating and hardening technique was reported for the preparation of highly satisfactory graphite-loaded epoxy-based voltammetric electrodes and the technique was applied to the preparation of disc and coated-wire electrodes. The surface that was to support the electrode was coated thinly and smoothly with a graphite-loaded epoxy base containing no hardener. The surface was then placed in a vapour chamber over 40% *m*/*m* hydrofluoric acid for a few minutes before being removed from the chamber and the epoxy coating was hardened at 50–60 °C by the hydrofluoric acid it had absorbed. The electrode surface was built up by repeating the process.

The coated wire electrodes were prepared from new copper wire. This was treated first with 5 M nitric acid solution and then with acetone and allowed to dry without washing it with water at any stage. A small amount of graphite-loaded epoxy was placed on the wire and smoothed with a tissue and then a thin flexible polythene or Cellophane sheet. The very thin and smooth layer that was produced was then hardened using the vapour hardening technique. Further hardened layers were built up by repeating the process.

To produce perfectly satisfactory graphite-coated wire electrodes it was found to be essential to cover the copper surface completely, as otherwise the copper surface affected the response. A particularly vulnerable place on the surface is the tip and it was recommended that whenever feasible this should be isolated from later contact with the electrolyte. An unloaded epoxy containing 40% *m*/*m* hydrofluoric acid as hardener was used to isolate the tip and also to seal the coated-wire electrode into a glass tube holder.

In the work described here, the multi-layer coating and vapour hardening technique has been extended to the production of coated plastic and glass rod electrodes. An advantage of using plastic or glass instead of wire is that it is not as critical to coat the surface as thoroughly because the plastic or glass is non-conducting. A disadvantage arises from this non-conductivity, however, in that coated plastic rod or coated glass rod electrodes produced in a similar way to the coated wire electrodes[1] have too high an electrical resistance. This difficulty has been overcome here by enriching the first few epoxy layers with graphite powder immediately after they have been hardened. Electrodes produced in this way have a sufficiently low resistance.

Experimental

Graphite-loaded Epoxy Base

The graphite-loaded epoxy was prepared by mixing 0.44 g of epoxy base (*i.e.*, the contents of the adhesive tube of a two-tube Araldite pack) (Ciba-Geigy) with 0.56 g of Specpure graphite, pelletable grade 1 (Johnson Matthey Chemicals), on a suitable plastic or glass surface. This mixture can be stored indefinitely in a suitable glass or plastic container.

Gaseous Hardener

The plastic rod or glass rod to be coated was treated with a thin layer of the graphite-loaded epoxy base as discussed later. A plastic screw-cap container was used as a vapour chamber. A small amount (2 ml) of 40% *m*/*m* hydrofluoric acid was placed in a plastic beaker, which in turn was placed in the container. Previously,[1] articles to be hardened were either suspended inside the container in a nylon net or suspended through small holes drilled in the cap. In this work an alternative method was also used. A holder for a plastic beaker was constructed from copper wire such that the holder could be hooked over the side of the container. Articles to be hardened were placed in the beaker in the container for 5 min to absorb hydrofluoric acid. The articles were then removed from the container and heated at 50–60 °C over a hot-plate.

Caution—Extreme precautions should be taken when using hydrofluoric acid. Work should be carried out in a fume-cupboard using the relevant safety equipment. Care must also be taken not to handle directly surfaces that may still retain some hydrofluoric acid.

Graphite-loaded Epoxy Resin

This was prepared immediately before use by mixing 1 drop (0.1 ml) of 40% *m*/*m* hydrofluoric acid with 0.5 g of graphite-loaded epoxy base prepared as above. The mixture hardened in about 40 min at room temperature, and was used to form electrically conducting bonds between conducting materials.

Isolating Epoxy Resin

This was prepared immediately before use by mixing 1 drop (0.1 ml) of 40% *m*/*m* hydrofluoric acid with 0.5 g of unloaded epoxy base. The mixture hardened in about 40 min at room temperature.

Preparation of Graphite-loaded Epoxy Plastic Rod or Glass Rod Electrodes

Plastic and glass rods (2–3 mm diameter) were used in 7-cm lengths. The plastic rods used were in fact knitting needles made of polystyrene. The surface of the rods was roughened using an abrasive paper (fine-grade abrasive, Acton and Borman); the ends of plastic rods were flattened using this abrasive paper. The surfaces were then wiped with a tissue,

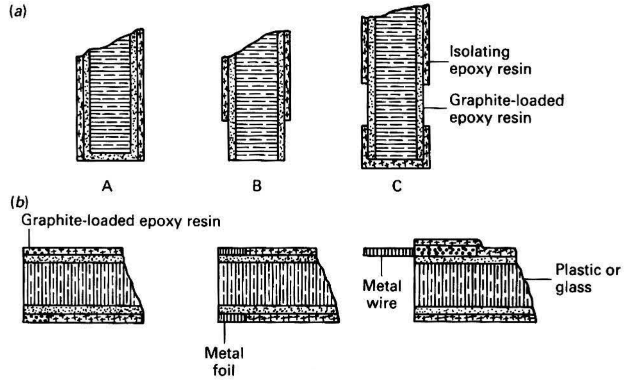

Fig. 1. (*a*) Types of coated rod electrodes: A, end surface active (disc); B, end cylinder, and C, central cylinder. (*b*) Methods of making electrical contact: A, with a graphite-loaded epoxy-resin coating; B, with metal foil; and C, with a metal wire bonded in place with graphite-loaded epoxy resin

washed with ethanol and dried. A very small amount of the graphite-loaded epoxy base was placed on the cylindrical surface and thoroughly smoothed over most of the length using a thin flexible Cellophane sheet. It was essential that only a thin film was left in order to obtain effective hardening. The coated rod was suspended in the vapour chamber over 40% *m/m* hydrofluoric acid for 5 min. The rods were then removed and hardened at 50–60 °C over a hot-plate for a further 5 min. For some types of electrode the end of the rod was also coated.

A small portion of Specpure graphite was placed on the hardened surface and smoothed into the layer using a tissue. The graphitised layer was then rubbed intensively with a tissue in order to remove the excess of graphite powder. The surface now had a shiny, mirror-like finish owing to the entrapment of graphite in the surface. The process of coating the surface with graphite-loaded epoxy and of hardening and graphitising the coating was repeated twice.

The coating and hardening process was then repeated twice more without the graphitisation process. The combination of three graphitised layers and two normal layers produces electrodes of low resistance (<150 ohm for a 6-cm length) with a sufficiently robust surface.

Three types of plastic and glass rod electrodes were prepared, as illustrated in Fig. 1(*a*). In type A both the cylindrical and end surfaces were coated with graphite, but the cylindrical surface was isolated subsequently from later contact with the electrolyte by coating it with the isolating epoxy resin. This electrode is thus a coated disc electrode. In types B and C the end surface was left uncoated. The type B electrode is essentially an end cylinder, the remainder of the cylindrical surface being treated with isolating epoxy resin; the end surface that is uncoated plastic was cleaned finally with an abrasive to remove any graphite particles. The type C electrode is of the central cylinder type. In this work the coated glass rod electrodes that were prepared were of types B and C.

Electrical contact was made at the end of the rod away from the electrode surface. Several methods of making contact were used, three of which are illustrated in Fig. 1(*b*). In type A the graphite coating at the end of the electrode was covered with graphite-loaded epoxy resin, which was allowed to harden. Electrical contact was then made very conveniently by means of a crocodile clip. In type B metal foil (*e.g.*, aluminium foil) was wrapped round the coated surface and was held firmly in position by some means; electrical contact was made to this, again possibly with a crocodile clip. In type C a copper wire was fixed to the coated surface by means of graphite-loaded epoxy.

Testing the Electrodes

The electrodes were tested in the static mode using slow (5–25 mV s^{-1}) linear sweep voltammetry and differential-pulse voltammetry. The three-electrode system consisted of the test electrode, a calomel reference electrode and a platinum counter electrode. Voltammetry was carried out by means of a Metrohm Polarecord 626, which incorporates its own recorder.

The background currents associated with the electrodes in a range of buffer solutions were investigated, and the oxidation of iodide, food colouring matters and dopamine was studied as examples.

Results

As an extension of previous work[1] on the production of graphite-loaded epoxy-coated wire electrodes, attempts were made to coat plastic rods in a similar manner. The resistance of a 6-cm length of plastic rod coated in the same manner even with eight to ten coatings was typically 1000 ohm or more. Using the present graphitisation procedure the resistance of similar electrodes with only five coatings was <150 ohm. Coated plastic and coated glass rod electrodes have an advantage over coated wire electrodes in that the material of the rod does not interfere with the response of the electrode. Thus, highly satisfactory electrodes can be made without the same degree of care to ensure that the original surface is covered adequately.

During the development stage in this work numerous plastic rod electrodes of the three types shown in Fig. 1(*a*) were prepared. All were readily produced and behaved similarly. Most of the later tests were carried out using the end-cylinder type and the results presented are for this type. Coated-glass rod electrodes behaved similarly. The coating and vapour hardening procedure worked particularly well on slightly roughened glass, presumably owing to the etching action of the hydrofluoric acid.

The electrodes behave very similarly to the graphite-loaded epoxy-coated electrodes described previously[1] and to well behaved glassy carbon electrodes. Three slow linear sweep scans from 0 to +1.2 V were used to condition the electrodes as previously.[1] Background currents associated with a typical conditioned electrode in pH 2 Britton - Robinson buffer, 0.18 M sulphuric acid, pH 3 ammonium citrate buffer and pH 7.5 Britton - Robinson buffer are shown in Fig. 2. In all these buffers without deoxygenation the background current between −0.3 and +1.1 V was less than 0.2 μA. Typical linear sweep voltammograms for the oxidation of 10^{-3} M potassium

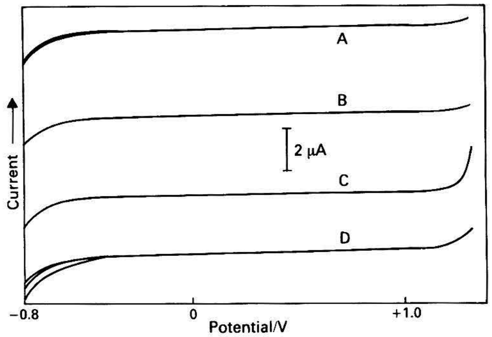

Fig. 2. Background currents (linear sweep voltammetry) for a conditioned graphite-loaded coated plastic rod electrode (end-cylinder type). No deoxygenation. Three successive scans in: A, pH 2 Britton - Robinson buffer; B, 0.18 M sulphuric acid; C, pH 3 ammonium citrate buffer; and D, pH 7.5 Britton - Robinson buffer. Scan rate = 10 mV s^{-1}

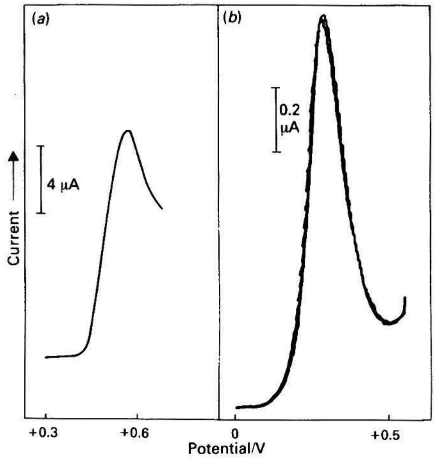

Fig. 3. Typical voltammograms at a graphite-loaded coated plastic rod electrode (end-cylinder type). (*a*) Four successive linear sweep scans (10 mV s^{-1}) without cleaning in a 10^{-3} M solution of potassium iodide in 10^{-1} M potassium chloride solution. (*b*) Three successive scans (differential-pulse voltammetry, 5 mV s^{-1}, 50 mV pulse height) of a 40 μg ml^{-1} solution of dopamine in pH 6.2 Britton - Robinson buffer. Cleaning between scans effected by placing electrode in 1.5 M sodium sulphite solution for 5 min at +1.5 V

iodide in 10^{-1} M potassium chloride solution are shown in Fig. 3(*a*); these show the excellent reproducibility of the signal without cleaning the electrode between scans.

At the levels of determinand used in static systems, contamination of the electrodes with the product of electrode reactions is a problem in many instances, as also occurs with other solid electrodes, including glassy carbon electrodes. Chemical and solvent cleaning of electrodes coated with graphite-loaded epoxy was discussed previously.[1] The same methods were used with the present electrodes. Glassy carbon electrodes are slightly easier to clean because harder physical pressure can be applied during the cleaning procedure. The effectiveness of a particular cleaning process depends on the nature of the adsorbed product. In this work an effective way of cleaning electrodes that had been used with dopamine was to place them in 0.1 M sodium hydroxide solution or 1.5 M sodium sulphite solution and hold them at a potential of +1.5 V for 5 min. The differential-pulse voltammograms for dopamine shown in Fig. 3(*b*) illustrate this point. Linear sweep voltammograms obtained with food colouring matters were similar to those obtained previously.[1]

Discussion

A method has been described for producing coated plastic and coated-glass rod voltammetric electrodes of low resistance and excellent performance. Their main advantage over coated metal wire electrodes is that complete coverage of the material of the rod is not essential. Indeed, in one type of electrode described here the plastic or glass end of the rod is left uncovered. The electrodes described are easy to construct and may be regarded as disposable. Coated wire electrodes in general, whether ion-selective or these new voltammetric electrodes, have the advantage of being simple to make, and there is the possibility of making them very small.

The graphite-loaded epoxy disc electrodes have so far been tested only briefly in flow systems but good results have been obtained and no difficulties in this use are envisaged. As with glassy carbon electrodes, contamination problems are expected to become minimal at the low determinand concentrations that are measured in flow systems.

A logical extension of the present work is the production of coated plastic and glass electrodes with other geometries. Indeed, the coating and graphitisation procedures described here should allow the production *in situ* of electrode surfaces on a wide range of electrically non-conducting surfaces of virtually any shape. A preliminary study has been made of the production of a thin-film voltammetric cell constructed from coated plastic plates, but further work is required to assess this. Work on the coating of carbon fibres, and indeed of plastic fibres and glass fibres, for possible use *in vivo* pharmacological applications is also being investigated.

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Polarographic Determination of Nitroxazepine Hydrochloride in Tablets*

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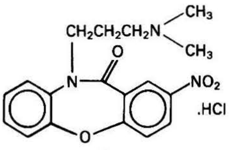
A polarographic method has been developed for the quantitative determination of nitroxazepine hydrochloride in the pure form and in tablets by the aid of different buffer systems. The substance is extracted from the sample with water, the appropriate buffer of selected pH is added to an aliquot and the solution then polarographed at a dropping mercury electrode *versus* a standard calomel electrode. The resultant single reduction wave is well developed and permits a precise quantitative determination. The method of standard additions is used.

Keywords: *Nitroxazepine hydrochloride determination; d.c. polarography; dosage forms*

Nitroxazepine hydrochloride, 10-[3-(dimethylamino)propyl]-2-nitrodibenz[*b*,*f*][1,4]oxazepine-11(10-*H*)-one hydrochloride (**I**), is an efficient tricyclic antidepressant drug endowed with a high therapeutic ratio that is used in the treatment of patients with depressive mood disorders.



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A review of the literature reveals that no attempt has been made to study the polarographic behaviour of nitroxazepine hydrochloride. However, several reports pertaining to the polarographic assay of different tricyclic antidepressants with phenothiazine rings[1–3] are available.

The purpose of this work was to establish the experimental conditions that permit the study of the polarographic behaviour of nitroxazepine hydrochloride and its determination in tablets.

Most of the work was performed under purely aqueous conditions, but as the addition of an aqueous solution shifts the half-wave potential of an electroactive ion to either a more positive or negative potential,[4–6] various non-aqueous solvents such as methanol, ethanol and propanol were used in order to study the effect of mixed-solvent composition on the half-wave potential ($E_{\frac{1}{2}}$) of nitroxazepine hydrochloride.

Experimental

Apparatus and Conditions for Polarographic Analysis

A manual polarograph S (Adept Laboratory, Poona) in conjunction with a spot galvanometer was used for the current-voltage measurements. A two-electrode combination was used consisting of a saturated calomel electrode (S.C.E.) and a dropping mercury electrode (D.M.E.). All the measurements were performed at 25 ± 0.2 °C. The D.M.E. had the following characteristics (in distilled water at 0.0 V open-circuit potential): $m^{2/3}t^{1/6} = 1.98$ mg$^{2/3}$ s$^{-\frac{1}{2}}$ at a mercury column height (h) of 50 cm and an applied potential range of 0.0–1.20 V.

Controlled-potential Electrolysis

A modified H-type Lingane cell,[7] with mercury pool cathode, platinum wire gauze electrode and spot galvanometer, was used for the controlled-potential electrolysis.

Suitable volumes ranging from 25 to 40 ml of buffer of selected pH (at which a well defined wave was obtained) were placed in each of the two compartments. The solution in the electrode compartment was deaerated with a stream of O_2-free nitrogen for 10 min. An appropriate volume of purified mercury was placed at the bottom of the working electrode compartment and the applied potential was set, usually at 1.00 V for each of the samples, and the corresponding current decay was noted using a galvanometer.

The galvanometer was then disconnected from the cell and a known volume (25 ml) of nitroxazepine hydrochloride stock solution was added to the working electrode compartment and deaerated. The galvanometer was turned on again and electrolysis was allowed to proceed virtually up to completion (*ca.* 4 h). The corresponding current decay was plotted against time.

Reagents and Solutions

All of the chemicals used were of either AnalaR grade from BDH Chemicals or general-reagent grade from E. Merck.

Four different buffer systems, namely acetate (pH 3.60–5.60),[8] McIlvaine (pH 2.20–8.00),[9] borate (pH 7.80–10.00)[10] and Britton - Robinson (pH 2.00–12.00),[11] were prepared in distilled water. A stock solution (10^{-3} M) of nitroxazepine hydrochloride was also prepared in distilled water. A 0.2% aqueous solution of Triton X-100 was used[12] to eliminate the polarographic maxima encountered throughout the polarogram.

General Procedure for Studying Polarographic Behaviour

Nitroxazepine hydrochloride (99.5%) was obtained from Hindustan Ciba-Geigy Ltd., Bombay, (Sintamil) and was used without further purification.

A 1.0-ml volume of the stock solution of nitroxazepine hydrochloride was taken in a polarographic cell, 0.1 ml of Triton X-100 and 8.9 ml of the appropriate buffer of selected pH were added and the solution was purged with O_2-free nitrogen for 10 min prior to each run. The stream of nitrogen was allowed to flow gently on the surface of the solution during the electrode reaction. The selected pHs were as follows: acetate, 4.00; McIlvaine, 4.60; borate, 8.00; and Britton - Robinson buffer, 5.00

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pH Dependence Studies

The polarograms of nitroxazepine hydrochloride were obtained in each of the four buffer systems taken over the entire pH range and the optimum pH range, which gave a well defined wave for each sample, was also found.

Effect of Mixed-solvent Composition

To study the effect of mixed-solvent composition on the half-wave potentials of nitroxazepine hydrochloride varying percentages of methanol, ethanol and propanol were added separately to the polarographic test solution and the polarograms were recorded after deaeration for 15 min.

Analysis of Tablets

Twenty tablets were weighed and the average mass per tablet was determined. A portion of the finely ground sample, containing 25–40 mg of nitroxazepine hydrochloride, was accurately weighed and transferred into a 100-ml calibrated flask containing 75 ml of distilled water. The contents of the flask were shaken for at least 20 min on a magnetic stirrer and then diluted to the mark with water. The solution was next filtered through a fine-pore filter-paper, discarding the first 20 ml of the filtrate. A 5-ml aliquot of the clear filtrate was pipetted into a 50-ml calibrated flask, 0.5 ml of Triton X-100 was added and the solution again diluted to the mark with the respective buffer of selected pH, as previously described. A 10-ml volume of this solution was injected into a polarographic cell and polarograms were recorded for a 0.0–1.20 V applied potential at a D.M.E. *versus* S.C.E.

After obtaining the polarograms, 1.0 ml of the standard solution (0.5 mg ml^{-1}) of nitroxazepine hydrochloride was added to the cell, deaerated for 2 min and again polarograms were recorded under the same conditions. The wave heights H and h were measured and the mass of nitroxazepine hydrochloride per tablet was calculated using the following equation[13]:

Mass of nitroxazepine hydrochloride per tablet (mg) =

$$\frac{ahb \times 1000}{(1.10H - h)W} \quad \ldots \ldots \quad (1)$$

where a is the mass of nitroxazepine hydrochloride reference standard in 100 ml of standard solution (mg); b is the average mass of a tablet (g); W is the mass of sample (mg) taken for the polarographic determination; h is the wave height of nitroxazepine hydrochloride before standard additions; H is the wave height of nitroxazepine hydrochloride after standard additions; and 1.10 is the dilution factor.

Recovery Experiments

In order to establish the reliability and suitability of the proposed method, known amounts of the pure drug were added to various pre-analysed formulations of nitroxazepine hydrochloride and the mixtures were analysed by the proposed method.

Interference Studies

The polarograms of the drug and suitable amounts of pharmaceutical adjuvents used in the tablet formulations, *i.e.*, starch, microcrystalline cellulose, lactose, talc and magnesium stearate, were also recorded in order to study the possible interference of excipients on the nature of the wave.

Results and Discussion

The determination and study of the electrochemical behaviour of most of the tricyclic antidepressants containing a phenothiazine ring reported so far, either involved an indirect determination[3,14] or utilised the oxidative property of the phenothiazine ring at a dropping mercury or rotating platinum electrode[2,15] whereas, in this work, nitroxazepine hydrochloride (an oxazepine derivative) gave a single, four-electron, reduction wave, which may be assigned to the facile reduction of the nitro group at position 2, giving a characteristic $E_{\frac{1}{2}}$ in all the buffer systems, namely acetate (pH 3.60–5.60), McIlvaine (pH 2.20–8.00), borate (pH 7.80–10.00) and Britton - Robinson (pH 2.00–12.00) over the entire pH range. A well defined wave was observed in each of these buffers in certain pH ranges (Table 1).

The nature of the wave was found to be diffusion controlled in the buffer systems taken, as shown by the linear dependence of limiting current on $\sqrt{h_{corr}}$ and [depolariser], constancy of the wave height in the pH range studied and the fact that di/dT had a very low temperature coefficient. The irreversible nature of the wave was confirmed by logarithmic plots.[16] The slope value of the plot of $E_{d.e.}$ *versus* $(i/i_d - i)$, which appreciably exceeded 59.2/n mV, and the numerical value of $E_{\frac{3}{4}} - E_{\frac{1}{4}}$ of the polarographic wave (appreciably exceeding 56.4/n mV) confirm the irreversible[17] nature of the wave.

The $E_{\frac{1}{2}}$ of nitroxazepine hydrochloride was dependent on pH and shifted towards more negative potentials with increase in pH of the buffer systems. Fig. 1(*a*) and (*b*) show the graphs of $E_{\frac{1}{2}}$ *versus* pH for all the buffer systems taken and a straight line is observed in each example.

The value of αn_a (Table 1) was determined by the method of Oldham and Perry,[18] where αn_a is the product of the transfer coefficient and number of electrons per molecule of the reactant involved in the rate-determining step of the electrode process. The value of P (number of protons involved per molecule of the reactant in the rate-determining step) was also determined[19] (Table 1).

An attempt to estimate n_a, the number of electrons involved in the rate-determining step, gave a value of 2 because for totally irreversible systems, as in this instance, α should be less[20] than 0.5. However, according to Meites[21] only a single electron can be transferred at a time during the course of the electrode reaction, and a value of n_a exceeding 1 should merely mean that successive steps are too close together to be distinguished on the time scale implicit in the polarographic measurements. However, the total number of electrons involved in the reduction process of nitroxazepine hydrochloride was found to be four, as determined by the controlled-potential electrolysis.

Table 1. Polarographic characteristics of nitroxazepine hydrochloride in various buffer systems. $c = 10^{-4}$ M

Buffer	Optimum pH range*	Selected pH†	$E_{\frac{1}{2}}$/V	i_d/μA	$dE_{\frac{1}{2}}$/dpH	αn_a	P
Acetate	4.00–5.00	4.00	0.38	0.374	0.077	0.96	1.26
McIlvaine	4.50–5.50	4.60	0.39	0.395	0.074	0.95	1.20
Britton - Robinson ..	4.00–5.00	5.00	0.43	0.384	0.074	0.95	1.20
Borate	7.80–8.60	8.00	0.59	0.291	0.076	0.90	1.16

* The pH range giving a well defined reduction wave.
†The pH at which the effects of various parameters were studied.

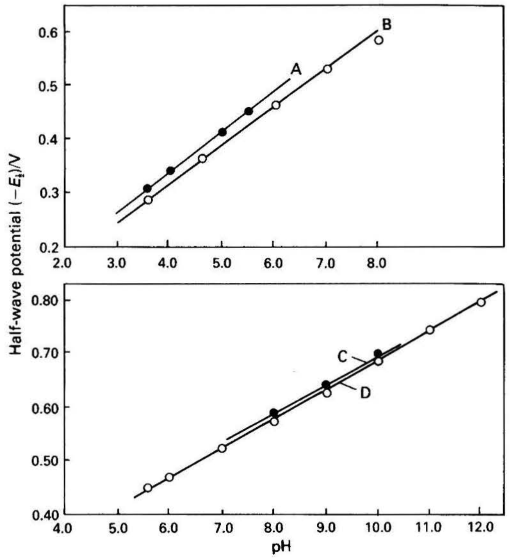
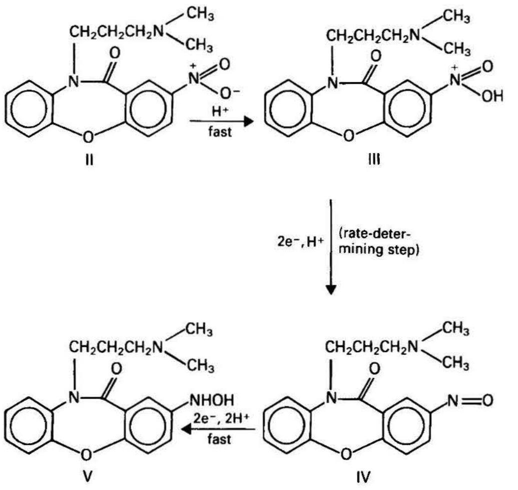


Fig. 1. Effect of pH on the half-wave potential of the nitroxazepine hydrochloride reduction wave in the buffer systems taken at $c = 1.0 \times 10^{-4}$ M. A, Acetate buffer; B, McIlvaine buffer; C, borate buffer; and D, Britton - Robinson buffer

After establishing the stoicheiometry of the rate-determining step, *i.e.*, $n_a = 2$ and H^+ (number of protons taking part) = 1, the following mechanism can be suggested for the polarographic reduction of nitroxazepine hydrochloride, which corresponds to the usual reduction mechanism[22] for the nitro group. The total four-electron reduction process represents the reduction of nitroxazepine hydrochloride to the corresponding phenylhydroxylamine derivative.



An increase in the percentage of methanol, ethanol and propanol in the polarographic test solution shifted the half-wave potentials towards a more negative potential with simultaneous decrease in diffusion current (Table 2). It should be noted that with propanol, it was not possible to study the effect at concentrations above 20% of alcohol, as the wave became distorted. However, at lower concentrations of propanol a significant negative shift in comparison with methanol and ethanol from −0.38 to −0.43 V *versus* S.C.E. in McIlvaine (pH 4.60) buffer was observed. An increase in the organic solvent content resulted in a rise in pH[23,24] and an increase in the dissociation constant of the protonated species.[25] Both of these factors lower the rate of protonation and consequently lead to a shift in $E_{\frac{1}{2}}$ of the reduction wave towards a more negative potential in all such situations where protonation proceeds electron transfer.

The decrease in diffusion current may be partly due to an increase in the viscosity of the medium and partly to an ion-pair factor.[26] The ion-pair factor must be considered because a continuous decrease in the diffusion current was observed. It also appeared that the above factors are not the only ones responsible for the observed shift in $E_{\frac{1}{2}}$; for nitroxazepine hydrochloride the observed shift is greater than it should be owing to the change in pH and dissociation constant. This additional shift in $E_{\frac{1}{2}}$ may be ascribed to a decrease in adsorbability and hence surface concentration of the depolariser with an increase in the percentage of non-aqueous solvent in the aqueous - organic mixture.[27] A decrease in surface concentration would retard the electrode process resulting in a decrease in $E_{\frac{1}{2}}$ and i_d.

Table 2. Effect of increasing the percentage of non-aqueous solvents on $E_{\frac{1}{2}}$ of nitroxazepine hydrochloride in McIlvaine buffer. $c = 10^{-4}$ M; pH 4.60

Ethanol, %	$E_{\frac{1}{2}}$/V	i_d/μA	Methanol, %	$E_{\frac{1}{2}}$/V	i_d/μA
20	0.40	0.367	20	0.41	0.357
30	0.42	0.336	30	0.43	0.326
40	0.44	0.306	40	0.46	0.295
50	0.45	0.285	50	0.48	0.275
60	0.45	0.275	60	0.48	0.265

Table 3. Assay of nitroxazepine hydrochloride tablets by d.c. polarography

Buffer	Labelled amount/mg	Amount found by proposed method*/mg	Recovery by proposed method, %	Standard deviation	Coefficient of variation, %
Acetate (pH 4.00)	25	24.7	99.8	0.0158	0.0633
	75	74.9	99.7	0.0186	0.0248
McIlvaine (pH 4.60)	25	24.9	101.2	0.0126	0.0504
	75	74.9	99.9	0.0134	0.0178
Borate (pH 8.00)	25	24.6	99.3	0.0164	0.0658
	75	74.5	99.2	0.0206	0.0274
Britton - Robinson (pH 5.00)	25	24.9	99.8	0.0158	0.0632
	75	74.9	99.7	0.0164	0.0218

* Each value is the average of five determinations.

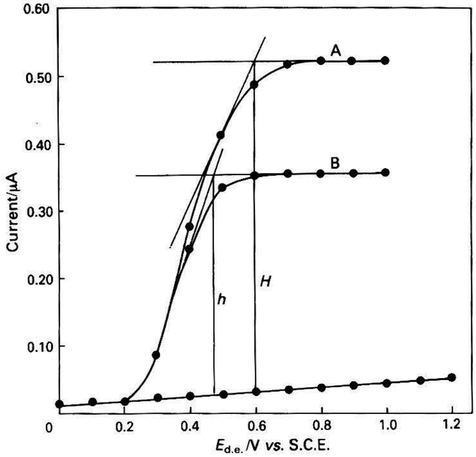


Fig. 2. Polarographic waves of nitroxazepine hydrochloride in McIlvaine buffer (pH 4.60) A, before and B, after the addition of the standard solution (0.5 mg ml^{-1})

Table 3 gives the results of the assay of the pharmaceutical dosage forms in all of the four buffer systems at their selected pH. The best results are observed with McIlvaine (pH 4.60) and Britton - Robinson (pH 5.00) buffers for both 25- and 75-mg tablets. In all four buffer systems analysis of the dosage forms is best performed in a less acidic pH range (pH > 3.60). With a more acidic medium, it is better to use a smaller percentage of non-aqueous solvents. However, in these analyses small percentages of only ethanol and methanol gave good results.

Fig. 2 shows the polarograms of the extracted drug, before and after addition of the standard solution of pure nitroxazepine hydrochloride. The method of standard additions is preferred because it is more rapid than a concentration - diffusion current plot method. Care should be taken with the size of the standard additions, because it influences the relative error of the result. However, there is an upper limit of concentration,[28,29] which may vary considerably from one substance to another, because above this the wave height of a substance is no longer proportional to its concentration. Moreover, the best result is observed if the standard addition is large.[30]

None of the excipients commonly employed in the tablet dosage form of nitroxazepine hydrochloride were found to interfere with the assay of the drug. Apparent variations of i_d/c can be produced by the potential impurities (if present) and react with the electroactive substance actually responsible for the wave. These are only apparent because it is actually c that is affected in each instance, while the diffusion current may be accurately proportional to the concentration of the electroactive substance that remains.[31]

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Determination of Lead, Mercury and Cadmium by Liquid Chromatography Using On-column Derivatisation With Dithiocarbamates

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The combination of HPLC with direct injection and on-column derivatisation provides a method for the multi-element identification of metals in pollution studies. However, problems have occurred when this technique has been applied to the determination of heavy metal ions. In this work the reasons for these difficulties has been studied and a modification of the procedure enabling lead, cadmium and mercury(II) ions to be determined is suggested.

Keywords: *Heavy metal determination; liquid chromatography; dithiocarbamates; on-column derivatisation*

In recent years there has been considerable interest in the use of normal- and reversed-phase liquid chromatography for the determination of metal ions as their chelates.[1,2] A range of different chelating groups have been used including dithizone, acetylacetone and the dithiocarbamates. In almost all of the studies the reagent was added in a pre-column reaction and the complex was extracted from the excess of reagent before analysis. It has been shown that for some metals a simpler procedure is to use a reversed-phase column and incorporate the reagent in the mobile phase.[3,4] The sample ions can be injected as an aqueous solution and the chelates are then formed by an on-column reaction before separation. The chelates can be selectively detected by either UV - visible[3] or electrochemical detectors[4] without interference from the excess of reagent in the mobile phase.

This combination of HPLC with on-column derivatisation and direct injection provides a potentially quick and easy method for the multi-element identification and determination of metals in pollution studies or in trade wastes as an alternative to inductively coupled plasma spectroscopy or repeated atomic-absorption measurements.

The technique has been successfully applied to the determination of copper, nickel, iron(III) and cobalt ions using sodium diethyldithiocarbamate (0.05%) as the reagent but lead and mercury ions gave poor peak shapes and low reproducibility of peak areas.[3] However, the pre-formed complexes of the heavy metals are apparently stable on reversed-phase chromatography as a number of workers have reported the separation of the dithiocarbamates of lead,[5–11] mercury,[6,8–13] and cadmium.[5–8, 11,13]

This paper describes a study of the reasons for the problems encountered in the determination of heavy metals and suggests a modification of the original on-column reaction technique by changing the mobile phase that enables lead, cadmium and mercury(II) ions to be determined following their direct injection as aqueous solutions.

Experimental

Reagents and Solutions

Metal salts. Reagent-grade lead nitrate, cadmium nitrate and mercury(II) chloride were obtained from Fisons Scientific Apparatus, Loughborough.

Dithiocarbamate. Sodium diethyldithiocarbamate was obtained from BDH Chemicals Ltd., Poole, Dorset.

Methanol and chloroform. HPLC grade from Fisons Scientific Apparatus, Loughborough.

Apparatus

Liquid chromatography was carried out using a Pye Unicam XPS pump connected to a column (10 cm × 5 mm) packed with ODS-Hypersil (Shandon Southern, Runcorn) and a Pye Unicam PU 4020 variable-wavelength detector operating at 350 nm. Samples (10 μl) were injected using a Rheodyne 7010 valve, into the mobile phase of methanol - water - chloroform (70 + 20 + 10) containing 0.05% *m/V* sodium diethyldithiocarbamate, which had a flow-rate of 1 ml min^{-1}. Some of the studies with cadmium used methanol - water - chloroform (55 + 40 + 5) containing 0.05% *m/V* sodium diethyldithiocarbamate.

Results and Discussion

In previous studies good linearity and repeatability were obtained from the determination of aqueous solutions of copper and other ions, but not for lead or mercury, by injection into methanol - water (80 + 20 *V/V*) containing 0.05% *m/V* sodium diethyldithiocarbamate.[3] The first aim of this study was, therefore, to locate the cause of this poor reproducibility in peak heights and shapes for the heavy metal complexes.

In earlier studies of pre-formed dithiocarbamate chelates by other workers some problems had also been encountered with this group of metals. Hutchins *et al.* suggested that the peak shapes were poor because of exchange reactions of the chelates with the nickel in the stainless-steel columns.[11] They tried to suppress this effect by the addition of EDTA to the mobile phase and the use of plastic-walled columns. However, the EDTA totally displaced cadmium and lead ions from their

Table 1. Separation of metal ions by reversed-phase HPLC by injection into a mobile phase containing dithiocarbamates

Metal ion	t_r/‡min	k'
Pb(II)*	4.8	3.80
Cd(II)*	3.7	2.70
Hg(II)*	7.2	6.20
Hg(I)*	5.9	4.90
Cd(II)†	4.2	3.2

* Mobile phase: methanol - water - chloroform (70 + 20 + 10) + 0.05% *m/V* sodium diethyldithiocarbamate.
† Mobile phase: methanol - water - chloroform (55 + 40 + 5) + 0.05% *m/V* sodium diethyldithiocarbamate.
‡ Retention time.

Table 2. Calibration graphs for peak areas against concentration of Pb(II) ions injected into a mobile phase containing sodium diethyldithiocarbamate. Mobile phase as in Table 1. Detection at 350 nm.

	Mean peak area/mm^2		
Concentration of Pb(II), p.p.m.	Area (attenuation)	Adjusted to 0.08	Coefficient of variation,* %
0.1	93 (0.005)	6	14.4
0.2	198 (0.005)	12	3.1
0.4	349 (0.005)	22	1.6
0.6	286 (0.01)	36	4.6
1.0	488 (0.01)	61	2.0
5.0	513 (0.04)	256	1.6
10.0	567 (0.08)	567	2.1

Correlation 0.9988
Slope 55.7
Intercept .. .. −0.6

* Results based on five replicate injections.

Table 3. Calibration graphs for peak areas against concentration of Hg(II) ions injected into mobile phase containing sodium diethyldithiocarbamate solution. Mobile phase as in Table 1. Detection at 350 nm

	Mean peak area/mm^2		
Concentration of Hg(II), p.p.m.	Area (attenuation)	Adjusted to 0.08	Coefficient of variation,* %
0.5	127 (0.005)	8	12.3
0.8	241 (0.005)	15	6.6
1.0	312 (0.005)	19	2.7
5.0	207 (0.04)	103	3.1
10.0	404 (0.04)	202	2.4

Correlation 0.9998
Slope 20.4
Intercept .. .. −1.0

* Results based on five injections.

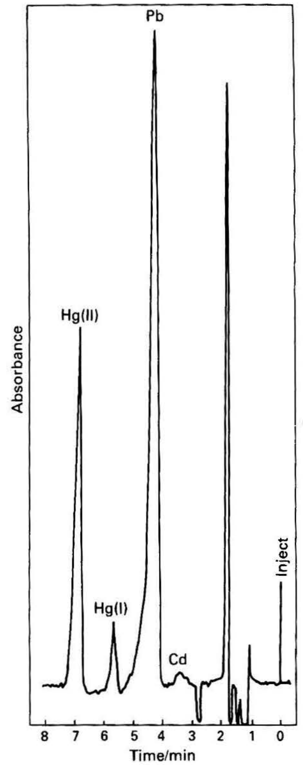

Fig. 1. Separation on an ODS-Hypersil column of chelates formed by the injection of 10 µl of a mixture containing 1 p.p.m. of lead, mercury(II), mercury(I) and cadmium ions into methanol - water - chloroform (70 + 20 + 10) containing 0.05% *m*/*V* sodium diethyldithiocarbamate as eluent. Detection at 350 nm and 0.01 a.u.f.s.

chelates and they were unretained. Other workers have used EDTA to prevent exchange reactions during the separation of mercury dithiocarbamates.[12] To test if exchange was occurring in this study samples of lead ions were left in the injector loop or the syringe needle for varying times before injection into the mobile phase of methanol - water (80 + 20) containing 0.05% *m*/*V* sodium diethyldithiocarbamate. In other studies different flow-rates were used to change the residence time of the chelate in the column. In each instance the results were effectively the same with no evidence of an interaction changing the peak shapes or reproducibility.

In a separate study the column was removed and the flow passed directly into the detector so that the system was effectively used in a flow injection mode. With this system the peak heights of the chelates formed from ten injections of 1000 p.p.m. lead nitrate solutions showed poorer reproducibility [coefficient of variation (c.v.) 8.1%] than azobenzene (c.v. 1.7%), which was used as a neutral non-reactive test sample. It appeared, therefore, that it was the derivatisation step rather than the column that was causing the problems. Either the reaction was not continuing to completion or the product was being partially precipitated, causing peak broadening and poor reproducibility.

Drasch and co-workers found that injection of pre-formed lead and cadmium diethyldithiocarbamates on to an octylsilyl bonded silica column gave severely tailing and misshapen peaks with methanol - water (70 + 30) as the mobile phase.[5,6] As these chelates are readily soluble in organohalogen solvents they examined methanol - water - chloroform (50 + 25 + 25) as a possible mobile phase and good peak shapes and separations on the octylsilyl bonded silica column were obtained. They also successfully used methanol - water - chloroform (60 + 32 + 8) with an octadecylsilyl bonded silica column. Other laboratories reporting the separation of pre-formed chelates have also used unusual mixtures of solvents for the mobile phase including acetonitrile - water - ethyl acetate - 0.05 M sodium dithiocarbamate (60 + 24 + 5 + 5),[7] acetonitrile - water - 0.01 M ammonium tetramethylenedithiocarbamate (69 + 31 + 0.15)[8] and methanol - water - diethyl ether - pH 7 buffer - ammonium hexamethylenedithiocarbamate (82 + 9 + 3 + 3 + 3).[13]

As it seemed that the low solubility of the the heavy metal chelates in methanol - water mixtures might be the source of the poor results, the effect of using different eluents, containing less polar organic modifiers, was studied. Acetonitrile - water (70 + 30) plus sodium diethyldithiocarbamate and an ODS-Hypersil column gave good results for 100 p.p.m. solutions of lead (c.v. 2–3%) but much poorer results with 10 p.p.m. solutions. When methanol - water - chloroform (70 + 20 + 10) containing 0.05% *m*/*V* sodium diethyldithiocarbamate was studied the injection of lead ions gave a sharp peak for the chelate ($k' = 3.8$) (Table 1). The peak areas were much more reproducible than earlier (1 p.p.m. lead ions, c.v. 2.0%) and gave a linear calibration graph from 0.1 to 10 p.p.m. with good reproducibility (Table 2). The lower limit was set by a deterioration in reproducibility. Detection was carried out at 350 nm to reduce interference from the excess of reagent in the eluent. As the samples were injected in aqueous solution rather than the mobile phase, there was a major disturbance of the base line from $k' = 0$–2 because of solvent depletion effects. Because of this it was not possible to reduce the sample retention times significantly by using a stronger eluent.

As this mobile phase gave satisfactory results for lead ions, mercury(II) ions were also examined. The injection of mercury(II) chloride gave a sharp peak at $k' = 6.2$ and repeated injections gave reproducible retentions and peak areas. The calibration graph was linear from 0.5 to 10 p.p.m. of mercury(II) ions (Table 3). It was of interest to determine if the system could distinguish mercury(I) from mercury(II). The injection of a saturated solution of mercury(I) sulphate gave a peak at $k' = 4.9$ well resolved from the mercury(II) peak. However, the low solubility of mercury(I) compounds made the preparation of a calibration graph difficult and no further studies were carried out.

As expected from the spectra of the chelates the extension of the technique to cadmium ions was much less successful because the chromophore of the cadmium chelate is very similar to that of the dithiocarbamate reagent. There is only a small change in absorption at 350 nm on the formation of the chelate resulting in a much lower sensitivity than for the other metals studied. The retention time of the cadmium chelate was very short ($k' = 2.70$) and suffered interference from the base-line disturbance caused by the sample solvent. A longer retention time ($k' = 3.2$) and better peak shapes with freedom from interference could be obtained by using methanol - water - chloroform (55 + 40 + 5) plus dithiocarbamate as the mobile phase. However, the response was still very poor and using the maximum sensitivity range of the detector a 5 p.p.m. cadmium solution gave a similar peak area to a 0.03 p.p.m. lead solution. On increasing the cadmium ion concentration from 5 to 100 p.p.m. there was a marked increase in peak width from 0.2 to 1.2 min but only a small change in peak height, although the peak shapes were symmetrical. The relationship of the resulting peak areas with cadmium concentration was non-linear. This may be caused by a kinetic or slow mixing effect rather than sample precipitation but was not studied further.

A mixture of 1 p.p.m. of the four heavy metal ions could be readily resolved (Fig. 1) and the elution order corresponded to that found for the pre-formed complexes.[10] The sensitivity for lead and mercury(II) is similar to that of flame AAS but cadmium is probably too poor for effective use. In order to test if the method could be suitable for multi-element analysis a sample of trade waste was examined. It was found to contain 1.7 p.p.m. of lead and <1 p.p.m. of cadmium by HPLC compared with the atomic-absorption spectroscopic analysis of 2.1 p.p.m. of lead and 0.19 p.p.m. of cadmium.

Conclusion

The simple and rapid on-column derivatisation method with the modified eluent can, therefore, be used for the direct separation and determination of lead and mercury ions at the parts per million level but reagent interference and low sensitivity mean that it would not be suitable for cadmium ions.

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Determination of the Herbicides Frenock and Dalapon in Soil and River Water by Mass Fragmentography

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A method is described for determining frenock and dalapon in environmental samples such as river waters and soils, based on the reaction of 1-benzyl-3-*p*-tolyltriazene with an extract of frenock or dalapon from strongly acidified sample solutions to form benzylated species, which are subsequently analysed by mass fragmentography (with soil samples, steam distillation is applied prior to the extraction).

Apiezon L grease - phosphoric acid serves as the stationary phase in GC and the molecular ions with m/z = 236 and 232 are used to monitor frenock and dalapon, respectively. The detection limits for frenock and dalapon are 0.05 and 0.5 ng, respectively, with 5 µl of sample injected. Recovery experiments using actual river water and soil samples showed recoveries of more than 92% with a coefficient of variation of less than 5% (n = 7). The method is capable of the simultaneous determination of frenock and dalapon with sufficient sensitivity and selectivity to be applicable to environmental samples.

Keywords: *Frenock and dalapon determination; mass fragmentography; herbicide residues; soil; water*

Sodium 2,2,3,3-tetrafluoropropionate (frenock), a contact-type herbicide, is widely used in forestry, and sodium 2,2-dichloropropionate (dalapon), a permeation-spreading herbicide, is employed in orchards and fields. These herbicides have been used in large amounts and in wide ranges of sites and it is therefore desirable from the standpoint of environmental pollution control to establish a simple, precise method for the trace determination of herbicides.

A literature search revealed no reference methods for frenock but some chromatographic methods have been reported[1–6] for dalapon. Ermolaeva *et al.*[1] used a gas chromatograph with a flame-ionisation detector (FID-GC); Chmil'[2] and Frank and Demint[3] applied a gas chromatograph with an electron-capture detector (ECD-GC) to the methyl ester derivative, Cotterill[4] to the 1-butyl ester derivative and Van der Poll and De Vos[5] to the 3-phenylpropyl ester derivative; and Chalaya and Gorbonos[6] applied thin-layer chromatography separation (TLC) with bromophenol blue as the colour reagent. Application of these methods to frenock was tried but without success, as frenock, when converted into its acid form, is highly volatile in comparison with dalapon. Subsequently, as described here, a method was developed in which both frenock and dalapon were extracted from strongly acidic solutions and converted into benzylated species with a carboxylated esterifying agent, and then subjected to a determination by mass fragmentography (MF).

The proposed method has the advantages over the conventional methods for dalapon that frenock and dalapon can be determined simultaneously, the analytical procedure is easy to perform and the selectivity is so high that coexisting substances hardly influence the analytical results, thus making it possible to obtain accurate results even for environmental samples containing various foreign matter.

Experimental

Chemicals

Sodium 2,2,3,3-*tetrafluoropropionate* (*frenock*). Daikin Kogyo Co., Japan.

Sodium 2,2-*dichloropropionate* (*dalapon*). Tokyo Kasei Co., Japan.

1-*Benzyl*-3-p-*tolyltriazene*. Tokyo Kasei Co., Japan.

Diethyl ether. Suitable for detection of pesticide residues.

Anhydrous sodium sulphate. As for diethyl ether.

All other chemicals used were of guaranteed grade.

Instrumental

The gas chromatograph - mass spectrometer was a Model JMS-D300 from Japan Electron Optics Laboratory Co. (JEOL). A glass column of 2 m × 2 mm i.d., packed with Apiezon L grease - H_3PO_4 (5 + 2) on Chromosorb W (AW-DMCS) (60–80 mesh) was used.

The column temperature was programmed from 150 to 200 °C at 10 °C min^{-1}, the injection port temperature was 220 °C, the enricher temperature 220 °C and the ion source temperature 250 °C. The ionisation voltage was 30 eV and the ion multiplier voltage 2.5 kV. The carrier gas was helium at a flow-rate of 40 ml min^{-1}. The ions monitored were at m/z = 236 for frenock and m/z = 232 for dalapon.

Analytical Procedure

For aqueous samples

A 100-ml portion of the sample is placed in a 200-ml separating funnel, to which 35 g of NaCl, 6 ml of 9 M H_2SO_4 and 20 ml of diethyl ether are added. The funnel is shaken for 10 min and the aqueous layer is discarded. The ether layer is washed with 10 ml of saturated NaCl solution, dried with anhydrous Na_2SO_4 and transferred into a 100-ml flask. To this flask is added 1 ml of a 2% m/V solution of 1-benzyl-3-*p*-tolyltriazene in diethyl ether. The flask is held in a water-bath at 60 °C for 1 h to effect benzylation under refluxing conditions. After cooling, the solution is transferred, with 10–20 ml of diethyl ether, into a 100-ml separating funnel and washed with 10 ml of 1.2 M HCl and then twice with 10 ml of saturated NaCl solution. The ether layer is dried with anhydrous Na_2SO_4 and concentrated in a Kuderna - Danish apparatus to 2 ml, of which 5 µl is injected into the GC - MS system to be determined by MF.

For soil samples

About 20 g of soil are weighed into a distillation flask, to which 40 ml of water, 20 g of NaCl and 10 ml of 9 M H_2SO_4 are added.

The mixture is subjected to steam distillation until 180 ml of distillate have been obtained. Water is added to this distillate to give a volume of 200 ml, of which 100 ml are taken, followed by treatment as described above for aqueous samples.

It should be noted that a dry-mass correction is applied in such a way that a portion of sample is weighed in a stoppered weighing bottle and dried at 105–110 °C to constant mass in order to obtain the water content.

Results and Discussion

Selection of Extraction Conditions for Aqueous Samples

As pointed out by Frank and Demint,[3] both frenock and dalapon are extracted with difficulty unless the medium is strongly acidic and the NaCl concentration is high. The relationship between their recovery and the concentrations of both H_2SO_4 and NaCl were therefore studied.

First, to choose the H_2SO_4 concentration, 20 µg each of frenock and dalapon, 30 g of NaCl and different amounts of concentrated H_2SO_4 were added to 100 ml of distilled water to produce a final solution with an H_2SO_4 concentration of 0–1 M. Each solution was extracted with 20 ml of diethyl ether. A maximum and constant recovery was attained at 0.25 M H_2SO_4 for dalapon and at 0.5 M H_2SO_4 for frenock (see Fig. 1).

Next, to establish a suitable NaCl concentration, a similar experiment was carried out using 0–30% *m/V* NaCl in 0.5 M H_2SO_4. It was found that the recovery increased slightly at higher NaCl concentrations (see Fig. 2).

Hence a solution containing 0.5 M H_2SO_4 and saturated with NaCl is the optimum for the ether extraction.

Selection of Steam Distillation Conditions for Soil Samples

As in the extraction of aqueous samples, the yields of the steam distillation of both frenock and dalapon are affected by the concentrations of H_2SO_4 and NaCl. The following three experiments were, therefore, performed.

First, to decide the optimum amount of H_2SO_4, 20 g of soil, 50 ml of distilled water, 20 µg each of frenock and dalapon, 30 g of NaCl and different amounts of concentrated H_2SO_4 in the range 0–10 ml were placed in a distillation flask. The mixture was subjected to steam distillation until 200 ml of distillate had been obtained. As shown in Fig. 3, the recovery of frenock and dalapon increased with increase in the amount of H_2SO_4 added until it reached a constant value at 3 ml.

Next, to establish a suitable amount of NaCl, a similar series of NaCl solutions containing amounts in the range 5–50 g were prepared while the amount of H_2SO_4 was kept at 5 ml. The results (Fig. 4) demonstrated that constant recoveries of both frenock and dalapon were obtained when the amount of NaCl added was more than 15 g.

Another experiment was conducted to establish the optimum amount of distillate, in which 1 mg each of frenock and dalapon were added, the amounts of H_2SO_4 and NaCl were kept at 5 ml and 20 g, respectively, and the recovery was measured for 40-ml increments of the subsequent distillate. It was found that 160 ml of distillate gave more than a 95% recovery of dalapon, which has a lower distillation rate than frenock.

Hence the optimum steam distillation procedure was concluded to be as follows: to 20 g of soil sample were added 10 ml of 9 M H_2SO_4, 20 g of NaCl and 40 ml of distilled water, and the mixture was subjected to steam distillation until 180 ml of distillate had been collected.

Study of Conditions for Benzylation

The conditions for benzylation were examined with a view to reducing the polarity of the compounds in order to prevent tailing during chromatography, after selection of a high-boiling derivative of frenock and to increase the selectivity of

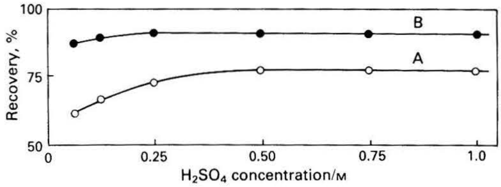


Fig. 1. Relationship between recovery and H_2SO_4 concentration for extraction from aqueous samples. A, Frenock; and B, dalapon

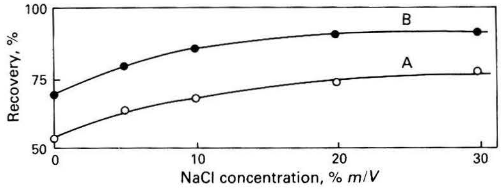


Fig. 2. Relationship between recovery and NaCl concentration for extraction from aqueous samples. A, Frenock; and B, dalapon

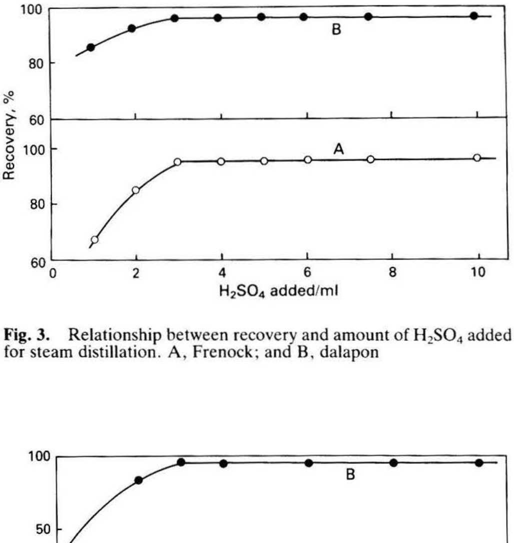
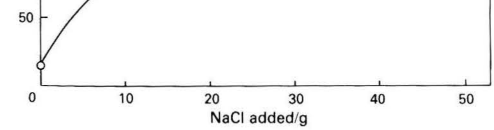


Fig. 3. Relationship between recovery and amount of H_2SO_4 added for steam distillation. A, Frenock; and B, dalapon



the monitored mass by increasing the relative molecular mass of the monitored species.

An experiment was carried out to determine the optimum volume of diethyl ether to be used in the benzylation: 20 μg each of frenock and dalapon were transferred into a 100-ml flask and 1 ml of 2% *m*/*V* 1-benzyl-3-*p*-tolyltriazene solution in diethyl ether and different amounts of diethyl ether in the range 5–40 ml were made to react by placing the flask in a water-bath at 60 °C for 1 h under refluxing conditions. As shown in Fig. 5, dalapon gave a constant yield when 10–40 ml of diethyl ether were added, whereas the yield of frenock was constant when 10–30 ml of diethyl ether were added and decreased with volumes over 30 ml.

Next, to decide a suitable reaction time, a similar experiment was conducted by keeping the amount of diethyl ether at 20 ml and varying the reaction time in the range 10–100 min. Fig. 6 indicates that the yield increased with increasing reaction time until a constant yield was obtained at 40 min. It was noted that the yield of frenock decreased if the reaction time was longer than 80 min.

Further, to investigate the relationship between the amount of benzylating agent added and the yield, 0.5 mg each of frenock and dalapon were taken, an amount of a 2% ethereal solution of 1-benzyl-3-*p*-tolyltriazene in the range 0.1–2 ml was added and the reaction was allowed to proceed under the above-specified conditions. A constant yield was obtained when a 2% ethereal solution of benzylating agent was used in amounts of more than 0.25 ml.

The procedure finally adopted was as follows: 20 ml of diethyl ether and 1 ml of 2% *m*/*V* 1-benzyl-3-*p*-tolyltriazene solution in diethyl ether were used and benzylation was effected by heating the system in a water-bath at 60 °C for 1 h. This procedure gave quantitative results when 2 mg each of frenock and dalapon were used.

Study of Conditions for GC - MS - MF

Several types of GC column packings were tested. The best separation was obtained with Apiezon L grease - H_3PO_4; other packings such as OV-17, SE-30 and DEGS can also be used.

To select monitor ions for the MF determination, benzylated frenock and dalapon were subjected to EI mass spectral measurement and the results obtained are shown in Figs. 7 and 8. Both benzylated frenock and dalapon gave their major fragment ion at m/z = 91, which corresponded to the benzyl group. However, this fragment ion was unsuitable because it is common to many other compounds found in environmental samples. For this reason, the derivative molecular ions ($M^{+\cdot}$) at m/z = 236 and 232 were selected as the monitor ions for frenock and dalapon, respectively.

To select the ionisation voltage capable of producing $M^{+\cdot}$ in the largest amount, voltages in the range 10–70 eV were tested. The most $M^{+\cdot}$ was produced at 30 eV for an ion source temperature of 250 °C.

The conditions selected were therefore Apiezon L grease - H_3PO_4 as the GC column packing, molecular ions $M^{+\cdot}$ with m/z = 236 and 232 as the monitor ions and an ionisation voltage of 30 eV.

Effect of Similar Substances

To detect any interferences in the determination, benzylations were tried on mono-, di- and trichloroacetic, propionic, butyric and valeric acid. In GC, dichloroacetic acid had almost the same retention time as dalapon, but gave no interference in the MF determination.

Calibration Graph and Recovery Experiments

To construct calibration graphs, standard frenock and dalapon in 100 ml of distilled water containing 0.5, 1, 2, 3, 4 and 5 μg of

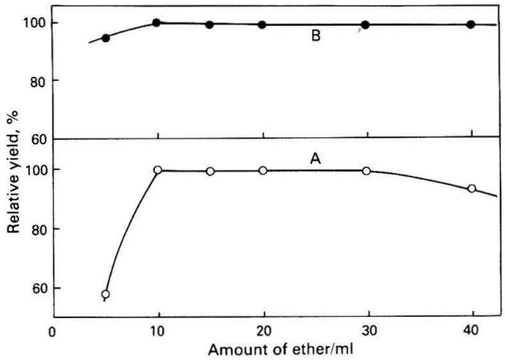


Fig. 5. Relationship between relative yield and amount of ether for benzylation. A, Frenock; and B, dalapon

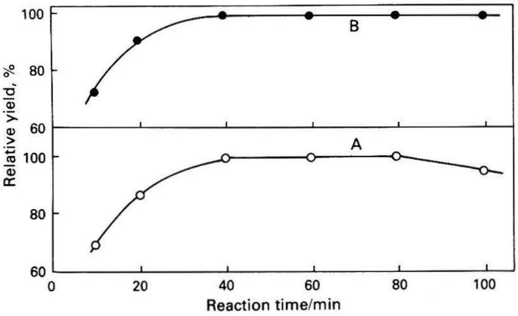


Fig. 6. Relationship between relative yield and reaction time for benzylation. A, Frenock; and B, dalapon

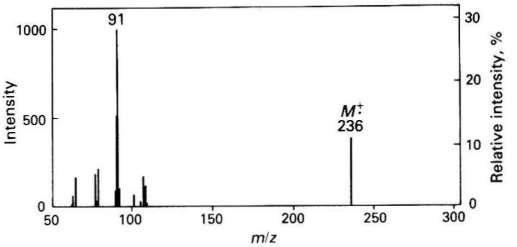


Fig. 7. EI mass spectrum for benzylated frenock. Column, 2 m, Apiezon L grease - H_3PO_4 (5 + 2); column temperature, programmed from 120 to 200 °C at 10 °C min^{-1}; injection port and enricher temperatures, 220 °C; ion source temperaure, 250 °C; ionisation voltage, 70 eV; and carrier gas, He at a flow-rate of 40 ml min^{-1}

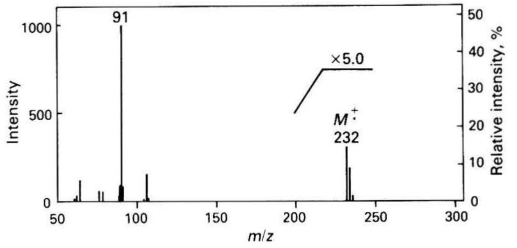


Fig. 8. EI mass spectrum for benzylated dalapon. Conditions as in Fig. 7

Table 1. Recovery of dalapon and frenock from river water and soil

Sample	Compound	Added/µg	Average recovery, %	Coefficient of variation, % ($n = 7$)
River water	Dalapon	5.0	96.8	2.5
	Frenock	1.0	97.0	3.1
Soil	Dalapon	10.0	93.5	4.7
	Frenock	2.0	92.7	3.6

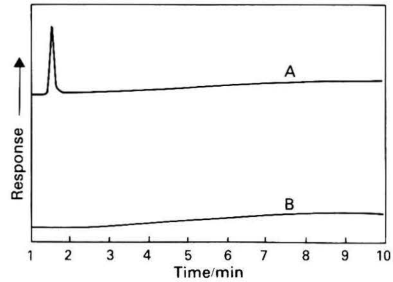


Fig. 9. Mass fragmentogram for a river water. Conditions as in Fig. 7 except column temperature, programmed from 150 to 200 °C at 10 °C min^{-1}, and injection port and enricher ionisation voltage, 30 eV. A, *m/z* 236 (frenock); and B, *m/z* 232 (dalapon)

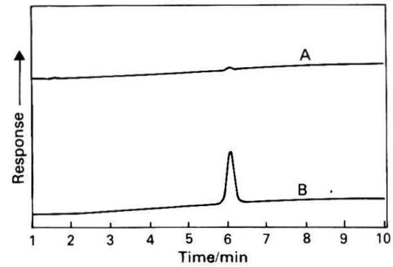


Fig. 10. Mass fragmentogram for a soil. Conditions and traces as in Fig. 9

each compound were subjected to the procedure described for aqueous samples. The MF calibration graphs for both frenock and dalapon were linear over the range examined. The detection limits were 0.05 ng for frenock and 0.5 ng for dalapon.

Recovery experiments were conducted by adding constant amounts of frenock and dalapon to 100 ml of river water or 20 g of soil without frenock and dalapon and carrying out the procedure as described for aqueous samples or soil samples, respectively. Based on the recoveries shown in Table 1, the proposed procedure was considered to be satisfactory, with recoveries of more than 92% and a coefficient of variation of less than 5%.

Application of the Procedure to Environmental Samples

The proposed method was applied to real samples of river water and soil. Fig. 9 shows the detection of frenock at 0.008 µg ml^{-1} in a river water sampled near a site where frenock had been released 1 week before. Fig. 10 shows the detection of dalapon at 0.23 µg g^{-1} in a field soil sample.

Conclusion

The procedure for the determination of frenock and dalapon can be summarised as follows. For extraction of aqueous samples the sample is saturated with NaCl and contains H_2SO_4 at a concentration of 0.5 M before being subjected to diethyl ether extraction. For steam distillation of soil samples 20 g of soil are mixed with 20 g of NaCl, 10 ml of 9 M H_2SO_4 and 40 ml of water and the mixture is subjected to steam distillation until 180 ml of distillate have been obtained. For benzylation 20 ml of diethyl ether and 1 ml of 2% *m/V* 1-benzyl-3-*p*-tolyltriazene in diethyl ether are added to the sample in a flask and the mixture is refluxed for 1 h. For MF determination molecular ions $M^{+\cdot}$ with $m/z = 236$ for benzylated frenock and 232 for benzylated dalapon are used as monitor ions. The detection limits are 0.05 ng for frenock and 0.5 ng for dalapon.

Recoveries from river waters and soils are more than 92% for both frenock and dalapon, with coefficients of variation less than 5%.

The proposed procedure is considered to be applicable to many other low relative molecular mass carboxylic acids, including halogenated compounds such as trichloroacetic acid.

We thank T. Okuhara, G. Yamaura and F. D. Chow for many helpful suggestions.

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Fluorimetric Determination of Tin at the Nanograms per Millilitre Level in Canned Beverages

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Diacetylmonoxime nicotinylhydrazone reacts with tin to produce a 2 : 1 fluorescent complex ($\lambda_{ex.}$ 420, $\lambda_{em.}$ 520 nm). A detailed study of the characteristics of this complex has been carried out and a fluorimetric method for the determination of tin at the 5–150 ng ml^{-1} level is proposed. The method has a detection limit of 2.1 ng ml^{-1}, a precision of about 1% on 70 ng ml^{-1}, is very selective and has been applied satisfactorily to the determination of tin in canned beverages without preliminary digestion of the sample.

Keywords: *Tin determination; fluorimetry; diacetylmonoxime nicotinylhydrazone; canned beverages*

Chemical reactions between canned foodstuffs and the tin container cause contamination of these foods. When the contamination reaches levels of over 200 µg ml^{-1} of tin, the organoleptic properties of the food can be seriously affected. Consequently, there has been much research carried out in order to obtain a rapid and specific analytical method for tin in canned products. Most of the procedures described require complete digestion of the samples or previous extraction of the tin using hydrochloric acid,[1] and both of these treatments are time consuming.

Several fluorimetric methods have been described for tin based on the formation of binary[2–10] or ternary[11] complexes and on the formation of ion association complexes,[12,13] but most of these methods have not been applied to the analysis of real samples.

In this paper a rapid and simple fluorimetric method for the determination of tin is described. It is based on the formation of a fluorescent complex with diacetylmonoxime nicotinylhydrazone (DMNH). The determination is very selective because only titanium, zirconium and hafnium cause serious interferences. It permits the determination of tin concentrations at as low as 5 ng ml^{-1}. To prove the applicability of this method, we have applied it to the determination of tin in canned beverages. The digestion of these samples is avoided and only the addition of sulphuric acid is necessary to obtain satisfactory results.

The DMNH reagent has been previously used to develop fluorimetric methods for titanium, zirconium and hafnium, which form ternary complexes with this hydrazone and inorganic anions.[14]

Experimental

Reagents

All experiments were performed with analytical-reagent grade chemicals and pure solvents.

Diacetylmonoxime nicotinylhydrazone (DMNH) solution, 5.5×10^{-4} M *in ethanol.* The synthesis and properties of this reagent have been described previously.[14]

Standard tin solution, 5.341 g l^{-1} *in* 9 M *sulphuric acid.* Prepared by dissolving the appropriate amount of granular tin in 50 ml of hot 9 M sulphuric acid. The solution was heated until the sulphuric acid fumed, cooled and diluted to 50 ml with distilled water. It was transferred into a 100-ml calibrated flask and diluted to the mark with 9 M sulphuric acid. For routine work a solution of 50 mg l^{-1} of tin in 1.5 M sulphuric acid was prepared from the standard solution.

Catechol violet solution.[15]

Apparatus

The fluorescence measurements were made with a Perkin-Elmer Model MPF-43A spectrofluorimeter with 1-cm quartz cells and a xenon-arc source. A set of samples of fluorescent compounds dissolved in a plastic matrix[16] was used daily to adjust the spectrofluorimeter to compensate for changes in the source intensity. Slit widths were adjusted to give a 6-nm band, in both the excitation and the emission monochromators.

Procedures

Determination of tin

In a 25-ml calibrated flask, appropriate volumes of the sample solution [giving a final concentration of Sn(IV) of between 5 and 150 ng ml^{-1}], 5 ml of 5×10^{-2} M phthalate buffer solution (pH 2.5) and 5 ml of 5.5×10^{-4} M DMNH solution were mixed. The solution was diluted to the mark with distilled water and placed in a constant-temperature bath at 20 °C for 20 min. The fluorescence intensity ($\lambda_{ex.}$ 420, $\lambda_{em.}$ 520 nm) was measured at 20 °C. The calibration graph was prepared by using standard solutions of Sn(IV) treated in the same way.

Preparation of samples

Tin has been determined in a variety of canned beverages. In all instances the pre-treatment required was as follows: 10 ml of concentrated sulphuric acid were added to 5 ml of sample in a 100-ml Erlenmeyer flask. The solution was diluted to about 75 ml with distilled water. Afterwards, it was cooled, filtered and washed with distilled water and the filtrate was collected in a 100-ml calibrated flask. A 0.1–0.2 ml aliquot of this solution was analysed according to the procedure described above.

Results and Discussion

Reaction of DMNH with Sn(IV)

Spectral characteristics

DMNH reacts with tin ions to give a fluorescent complex ($\lambda_{ex.}$ 420, $\lambda_{em.}$ 520 nm), the spectral characteristics of which are the same using Sn(IV) or Sn(II) solutions. Sn(IV) solutions were used in this study owing to the instability of Sn(II) solutions. The excitation and emission spectra are shown in Fig. 1. These spectra are uncorrected for emission characteristics of the light source or the response of the detector. The reagent solutions do not show fluorescent properties at any value of pH.[14]

Effect of different variables

The pH for optimum fluorescence intensity of the Sn - DMNH complex was investigated using hydrochloric, sulphuric and perchloric acids to control the acidity. The anion of the acid tested did not affect the maximum fluorescence intensity of the complex, in contrast to those of DMNH with titanium, zirconium and hafnium.[14] Only a binary complex of tin was detected. The optimum pH range was wider when sulphuric or perchloric acids were used ($1.9 < \text{pH} < 3.2$) than in the

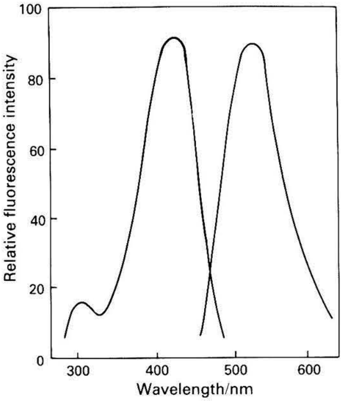

Fig. 1. Excitation and emission spectra of the tin - DMNH complex in phthalate buffer solution. [DMNH] = 10^{-4} M; [Sn(IV)] = 70 ng ml^{-1}; [buffer] = 10^{-2} M; pH = 2.5; and temperature = 20 °C

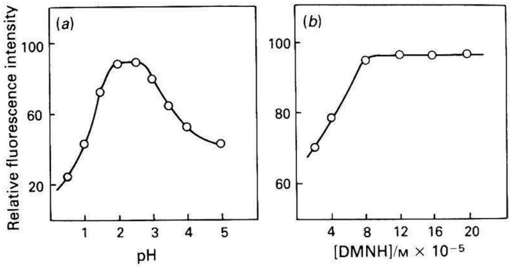

Fig. 2. Effect of (*a*) acidity and (*b*) DMNH concentration on the tin - DMNH complex

presence of hydrochloric acid ($2.1 < \text{pH} < 2.6$). This study was also carried out in the presence of phthalate and monochloroacetate buffers and the results were similar to those obtained with sulphuric or perchloric acids; however, the complex solution was more stable in the phthalate buffer solution. The variation of fluorescence intensity of the complex with pH in the presence of this buffer solution is shown in Fig. 2(*a*). The concentration of this buffer in the 2×10^{-3}–2×10^{-2} M range did not affect the development of the complex. A 10^{-2} M buffer solution of pH 2.6 was adopted.

The complex took 20 min to obtain its maximum fluorescence intensity, which remained constant for at least 4 h. The variation of the fluorescence intensity of the complex with DMNH concentration, in the 2×10^{-5}–2×10^{-4} M range, is shown in Fig. 2(*b*). A 1.1×10^{-4} M DMNH concentration was used thereafter. As DMNH is insoluble in water, the effects of three organic solvents on the fluorescence intensity of the complex were studied. Dimethylformamide and acetone caused a more negative effect than ethanol. Using 20% of ethanol in each sample a good fluorescent signal was obtained; at higher percentages the values were lower.

Several salts, NaCl, Na_2SO_4 and $NaClO_4$, were tested at different concentrations to study the effect of the ionic strength and the presence of several anions on the fluorescence intensity. None of these salts affected the system.

The effect of temperature on the fluorescence was studied over the 15–35 °C range. The tin - DMNH complex has a temperature coefficient of approximately −1% per °C. Therefore, the temperature should be controlled to within 1 or 2 °C.

To avoid Sn(IV) hydrolysis, it was necessary to add the buffer solution beforehand because if Sn(IV) and DMNH were mixed in the absence of the buffer, the fluorescence intensity decreased by 50%.

Table 1. Effect of various ions on the determination of 70 ng ml^{-1} of Sn(IV)

Ion added	Tolerance ratio of ion to Sn(IV)
Cr(III), Ni(II), Co(II), Be(II), Mg(II), Cu(II), Pt(IV), Zn(II), Cd(II), Al(III), Hg(II), Tl(I), Pb(II), Sb(III), Se(IV), Ce(IV), Fe(II), Ca(II), Sr(II), SO_4^{2-}, Cl^-, Br^-, NO_3^-, NO_2^-, CO_3^{2-}, AsO_2^-, S^{2-}, $S_2O_3^{2-}$	100
F^-, SCN^-, BO_2^-	75
MoO_4^{2-}, Ag(I), V(V), I^-	45
Bi(III), Cr(VI)	30
U(VI), Pd(II)	15
PO_4^{3-}, AsO_4^{3-}	3

Stoicheiometry of the complex

The stoicheiometry of the tin - DMNH complex was evaluated by the molar ratio and continuous variation methods. A metal to ligand ratio of 1 : 2 was found.

Spectrofluorimetric Determination of Tin with DMNH

Characteristics of the method

There was a linear relationship between the emitted fluorescence intensity and the tin(IV) concentration for between 5 and 150 ng ml^{-1} of Sn in the final solution. The detection limit (C_L) (as defined by IUPAC[17]) was determined for this procedure. For $n_B = 11$, a C_L value ($K = 3$) of 2.1 ng ml^{-1} was obtained. The relative standard deviation for 70 ng ml^{-1} of tin ($n = 11$, $P = 0.05$) was found to be ±1.1%.

Interference of foreign ions

By an identical technique to that used for the determination of tin, the effects of 44 ions on the proposed method were examined. The tolerance limits for these ions are given in Table 1, in which one can observe that 29 ions did not affect the determination of tin when they were present in 100-fold amounts. Only Ti(IV), Zr(IV) and Hf(IV) interfered when present at the same level as tin as they also form fluorescent complexes with DMNH under the given conditions. Fe(III) produced a serious negative interference because of the strong absorption of both the tin - DMNH fluorescence emitted and the exciting radiation by the yellow Fe(III) - DMNH complex. However, the reduction to the iron(II) ion with hydroxylammonium chloride permitted the direct determination of tin in the presence of 45-fold amounts of iron.

Applications

The method described was applied to the determination of tin in several samples of canned beverages. A series of tests has been carried out to develop a rapid and simple method for the determination of tin in this type of sample.

Initially, three different treatments were performed on the samples to find out if the complete elimination of the organic matter was necessary: in the first a complete digestion was effected using sulphuric - perchloric - nitric acids (1 + 1 + 3); in the second each sample was treated with 1.8 M H_2SO_4 (without heating); and in the third the samples were treated with 1.2 M hydrochloric acid (without heating). The samples from the first treatment were analysed directly and the other two required a preliminary filtration. The results obtained are summarised in Table 2. The organic matter from the samples treated with sulphuric acid was only partially removed but the results obtained agreed with those from the digested samples.

Table 2. Determination of tin in canned beverages with different treatments

	Tin found†/μg ml⁻¹		
Sample*	1	2	3
Pineapple juice	63.2 ± 0.7	66.1 ± 0.8	64.4 ± 0.8
Orange juice . .	34.1 ± 0.8	36.2 ± 0.6	5.1 ± 0.6
Lemonade . .	39.6 ± 0.9	35.5 ± 0.4	15.6 ± 0.9
Peach juice . .	84.3 ± 0.7	80.0 ± 0.8	20.4 ± 0.5
Orange nectar . .	82.6 ± 0.6	79.8 ± 0.5	27.6 ± 0.9
Pear nectar . .	76.4 ± 0.9	81.1 ± 0.7	5.6 ± 0.6

* Qualitative composition: pineapple juice (pineapple pulp, sugars, citric acid and water); orange juice (40% orange juice, saccharose, fructose, citric acid, ascorbic acid, β-carotene and water); lemonade (6% lemon juice); peach juice (peach pulp, sugars, citric acid and ascorbic acid); orange nectar (orange juice, sugars, citric acid, natural aroma of orange and water); pear nectar (pulp pear, sugars, citric acid and water).

† Average of six determinations. Treatments as follows: 1, digestion with 1 + 1 + 3 sulphuric - perchloric - nitric acids; 2, addition of concentrated sulphuric acid and filtration; and 3, addition of concentrated hydrochloric acid and filtration.

Table 3. Recovery of tin added to canned beverages

	Tin content/μg			
Sample	Added*	Found†	Expected	Recovery, %
Pineapple juice . .	—	330.0	—	—
	25	357.1	355.0	100.6
	50	379.3	380.0	99.8
	75	408.1	405.0	100.7
	100	425.8	430.0	99.0
Orange juice	—	180.0	—	—
	25	210.8	205.0	102.8
	50	230.0	230.0	100.0
	75	254.6	255.0	99.8
	100	277.5	280.0	99.1
Lemonade	—	177.5	—	—
	25	197.1	202.5	97.3
	50	232.5	227.5	102.2
	75	263.0	252.6	104.1
	100	268.5	277.5	96.7
Peach juice	—	400.0	—	—
	25	420.3	425.0	98.9
	50	455.0	450.0	101.1
	75	480.6	475.0	101.2
	100	495.0	500.0	99.0
Orange nectar . .	—	399.0	—	—
	25	420.2	424.0	99.1
	50	450.0	449.0	100.2
	75	473.1	474.0	99.8
	100	498.5	499.0	99.9
Pear nectar	—	405.5	—	—
	25	420.6	430.5	97.7
	50	456.0	455.5	100.1
	75	479.1	480.5	99.7
	100	505.0	505.5	99.9

* Tin added to 5 ml of the sample before the treatment with sulphuric acid.

† Average of six determinations by taking 0.1 and 0.2 ml of treated sample.

Table 4. Comparison of results for the analysis of different canned beverages by catechol violet and DMNH

	Tin found*/μg ml⁻¹	
Sample	Fluorimetric method	Standard method
Pineapple juice	45.7 ± 0.9	40.5 ± 0.7
Orange juice	61.5 ± 0.4	63.2 ± 0.9
Lemonade	24.3 ± 0.8	19.2 ± 0.6
Peach juice	77.8 ± 0.6	71.4 ± 0.7
Orange nectar	37.1 ± 0.7	40.5 ± 0.6
Pear nectar	95.7 ± 0.5	99.8 ± 0.8

* Average of three determinations.

The samples treated with hydrochloric acid gave lower results because in this treatment the whole of the organic matter remains in solution. From these tests it can be concluded that the simple addition of sulphuric acid to the samples is enough to obtain acceptable results and allow the rapid determination of tin in canned beverages.

To test the applicability of the method described using the treatment with sulphuric acid, various amounts of tin were added to each sample of canned beverage before the addition of sulphuric acid. The results obtained are shown in Table 3. The recoveries were calculated by comparing the results obtained before and after the addition of standard tin solutions. The data obtained show the method to be precise over a wide range. To obtain these data, a high dilution factor (because of the sensitivity) was used. This dilution prevents the residual organic matter of the samples from interfering, but avoids their total digestion. When hydrochloric acid was added to the samples, we obtained very different recoveries according to the amount of tin added. This observation shows that the matrix of the samples can cause interference in spite of the high dilution of the sample.

The results obtained by the proposed method for several samples were compared with those obtained by a standard colorimetric method,[15] based on the formation of a complex between tin and catechol violet. In the proposed method the preliminary treatment of the samples was carried out by the addition of sulphuric acid and by digestion with 1 + 1 + 3 sulphuric - perchloric - nitric acids (for destruction of organic matter, when the standard method was used). The data in Table 4 show a good correlation between the two methods. The samples analysed have the same qualitative composition as those presented in Table 2, but the concentrations of tin are different because a different set of samples was used.

It can therefore be concluded that the proposed method is more rapid and simple for the analysis of this type of sample than the standard method used previously.

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Spectrophotometric Determination of Phenyltrimethylammonium Benzenesulphonate and Its Ethoxide by Ion-pair Extraction

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A simple spectrophotometric method is described for the determination of microgram amounts of phenyltrimethylammonium benzenesulphonate and its corresponding ethoxide by ion-pair extraction with methyl orange. The molar absorptivities and standard error of the mean for the sulphonate and ethoxide at 420 nm are 58.90 l mol^{-1} cm^{-1} and 0.04–0.289% and 178.6 l mol^{-1} cm^{-1} and 0.033–0.289%, respectively.

Keywords: *Phenyltrimethylammonium benzenesulphonate determination; phenyltrimethylammonium ethoxide determination; methyl orange; ultraviolet spectrophotometry*

The methylation of morphine is an important operation in the opium alkaloids industry. About 90% of manufactured morphine is converted into codeine by a methylation procedure. Phenyltrimethylammonium ethoxide, obtained from the corresponding sulphonate by treatment with sodium ethoxide, is a popular methylating agent and is commonly used for the methylation of morphine. A reliable and rapid method for its determination is essential for the effective process control of codeine manufacture. The method hitherto adopted for its determination involves titration with standard acid. We felt that this procedure is inaccurate as both the quaternary ethoxide and the free sodium ethoxide will react with acid and thereby give higher analytical values for the quaternary ethoxide. Therefore, we were interested in developing a suitable method that can accurately determine the strength of the quaternary ethoxide in the presence of sodium ethoxide.

A survey of the literature revealed several methods for the determination of quaternary ammonium compounds.[1–5] The most frequent procedure adopted is spectrophotometry.[6,7] However, no suitable method has been reported for the determination of phenyltrimethylammonium benzenesulphonate and the corresponding ethoxide. We describe here a simple and accurate method based on ion-pair extraction with methyl orange indicator.

Many amines and quaternary ammonium compounds can be determined in aqueous solution by forming a salt or ion pair between the positively charged nitrogen atom and the negatively charged dye or indicator molecule, extracting this ion pair into an organic solvent and determining the concentration of the extracted dye spectrophotometrically. The pH of the aqueous phase is critical for the success of this method. The dye must be present in its anionic form so that it can form the ion pair with the quaternary nitrogen atom and the excess of reagent will remain in aqueous solution, unextracted by the solvent. The optimum pH range is usually near or above the pK_a of the acid dye. On the other hand, in the acidic pH region the amines exist as positively charged species owing to protonation of the nitrogen atom. Because quaternary ammonium compounds remain positively charged at the nitrogen atom at all pH values, it is possible to extract and determine them.[8–10] The proposed method for the determination of phenyltrimethylammonium sulphonate and its ethoxide is based on this unique property.

Experimental

Apparatus

A Perkin-Elmer Model 550S double-beam spectrophotometer and stoppered cells of 1-cm light path were used to record the absorbance. For pH measurements an electronic digital pH meter was used. All the reactions and analytical measurements were carried out at room temperature (*ca.* 25 °C).

Reagents

All reagents were of analytical-reagent grade unless otherwise stated. Glass-distilled water was used for the preparation of all the solutions.

Phenyltrimethylammonium benzenesulphonate solution, 0.01 M. This salt was prepared by reacting dimethylaniline with a slight excess of methyl benzenesulphonate in toluene and purified by repeated recrystallisation from ethanol containing a small amount of ethyl acetate.[11] A 0.01 M stock solution was prepared by dissolving 0.7325 g of the salt in 250 ml of water.

Methyl orange solution, 0.01 M. This was prepared by dissolving 1.6367 g of methyl orange in 500 ml of water.

Chloroform. Re-distilled laboratory-reagent grade chloroform was used.

Buffer solution (pH 9). This was prepared by mixing 50 ml of 0.2 M boric acid - potassium chloride (12.367 g of boric acid + 14.911 g of potassium chloride dissolved in 1000 ml of water) with 21.30 ml of 0.2 M NaOH and diluting with water to 200 ml.

Procedure

Measure 1 ml of the sample solution containing 300–1700 µg ml^{-1} of the salt into a conical flask. Add 10 ml of the buffer solution of pH 9 and then 3 ml of 0.01 M methyl orange solution. Homogenise the solution by shaking it gently and allow it to stand for 10 min for completion of ion-pair formation. Add 20 ml of chloroform from a burette and shake the mixture for 20 min in a mechanical shaker. Separate the organic layer and measure the absorbance at 420 nm against a reagent blank.

Effect of pH on Reaction

Salt solution (1 ml; 0.01 M) was placed in each of a set of eight conical flasks and 1 ml of water was placed in each of another set of eight flasks. Solutions of different pH (10 ml) were added to all the flasks, followed by 3 ml of 0.01 M methyl orange solution. The flasks were allowed to stand for 20 min, then chloroform (20 ml) was added and the flasks were agitated in a shaker for 10 min. The organic layer was separated and its absorbance was recorded at 420 nm against the corresponding blank solution. The absorbance was found to be constant in the pH range 8–11 and pH 9 was therefore selected for the analytical studies. Below pH 4 negligible absorbance was observed, indicating non-formation of an ion pair.

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Optimum Time for Completion of Ion-pair Formation

This was studied by varying the time of addition of chloroform to the reaction mixture from 5 to 60 min and keeping the time for extraction of the ion pair constant (10 min). The absorbance values were found to be constant in all instances, indicating that the reaction proceeds to completion in less than 5 min.

Effect of Phase Volume Ratio

Keeping all other experimental parameters constant, the amount of chloroform used for extraction was varied from 14 to 42 ml in steps of 7 ml. The absorbance was constant in all instances.

Effect of Time on Extraction

This was studied by varying the shaking time from 5 to 60 min. The absorbance values showed that the extraction is complete after 20 min.

Molar Ratio

Molar ratio method

Keeping the volume of the quaternary salt solution (0.01 M) constant (2 ml), the volume of the methyl orange solution (0.01 M) was varied from 1 to 8 ml in steps of 1 ml and the absorbance of the chloroform extract was recorded in each instance. The results indicated that an optimum amount of 4 ml of methyl orange solution (0.01 M) is required for 2 ml of the quaternary salt solution (0.01 M). Therefore, the molar ratio of the quaternary salt to methyl orange is 1 : 2.

Job's method of continuous variations

In this method the molar ratios of the components are varied by changing the concentrations of both the components while maintaining the total number of moles of the components constant. The volume of quaternary salt solution (0.1 M) was varied from 1 to 7 ml in steps of 1 ml and the volume of methyl orange solution (0.01 M) was varied from 9 to 3 ml, thereby keeping the total number of moles constant. The absorbances of the solutions were determined at 420 nm and the molar fraction of the salt was plotted against absorbance. The graph indicated that the ion pair is formed with a molar ratio of the quaternary salt to methyl orange of 1 : 2.

Calibration Graph

A calibration graph was prepared under the optimum conditions described above. The graph was linear and obeys Beer's law in the concentration range 300–1700 µg ml^{-1}. The average molar absorptivity at 420 nm is 58.90 l mol^{-1} cm^{-1} and the standard error of the mean (average of three determinations) varies from 0.040 to 0.289% (Table 1).

Determination of Phenyltrimethylammonium Ethoxide

The experimental conditions and reagents employed for the determination of the quaternary ethoxide were the same as described above for the sulphonate.

Buffer Solution

Buffer solution of pH 10 was prepared by mixing solutions of disodium tetraborate(III) (50 ml; 0.025 M) and NaOH (18.3 ml; 0.1 M).

Phenyltrimethylammonium Ethoxide Solution

The required amount of sodium was dissolved in absolute ethanol and the concentration of the sodium ethoxide solution was determined by acid titration. The quaternary ethoxide was prepared by reacting equimolar amounts of the sodium ethoxide and analytically pure crystalline phenyltrimethylammonium benzenesulphonate below 15 °C. The precipitated sodium benzenesulphonate was filtered off. This method ensured that there was no excess of unreacted sodium ethoxide. The concentration of the solution was determined by titration against standard acid (H_2SO_4). It was diluted with an appropriate amount of absolute ethanol to give a solution of concentration 0.01 M.

Table 1. Determination of trimethylphenylammonium benzenesulphonate

Sample No.	Amount present/µg	Amount recovered/µg	Recovery, %	Coefficient of variation, %	Standard error of the mean, %
1	300	300.0	100.0	0.50	0.289
		298.5	99.5		
		301.5	100.5		
2	500	500.0	100.0	0.29	0.166
		500.0	100.0		
		502.5	100.5		
3	700	700.0	100.0	0.43	0.250
		694.8	99.3		
		694.8	99.3		
4	900	900.0	100.0	0.08	0.046
		900.9	100.1		
		899.5	99.9		
5	1100	1100.0	100.0	0.13	0.077
		1097.5	99.8		
		1100.0	100.0		
6	1300	1300.0	100.0	0.37	0.216
		1304.8	100.4		
		1295.1	99.6		
7	1500	1500.0	100.0	0.19	0.110
		1495.1	99.7		
		1500.0	100.0		
8	1700	1700.0	100.0	0.07	0.040
		1701.4	100.1		
		1702.4	100.1		

Table 2. Determination of trimethylphenylammonium ethoxide

Sample No.	Amount present/μg	Amount recovered/μg	Recovery, %	Coefficient of variation, %	Standard error of the mean, %
1	100	100.0	100.0	0.50	0.289
		99.5	99.5		
		100.5	100.5		
2	500	498.0	99.6	0.11	0.066
		499.0	99.8		
		499.0	99.8		
3	900	902.0	100.2	0.06	0.033
		902.0	100.2		
		901.0	100.1		
4	1300	1305.0	100.4	0.22	0.126
		1300.0	100.0		
		1305.0	100.4		
5	1700	1710.0	100.6	0.29	0.167
		1705.0	100.3		
		1700.0	100.0		

Procedure

To 1 ml of the quaternary ethoxide solution (0.01 M) were added 10 ml of the buffer solution (pH 10) and 7 ml of methyl orange solution (0.01 M). The reaction mixture was allowed to stand for 15 min after homogenising the solution by gentle shaking. Chloroform (25 ml) was added and the mixture was agitated in a mechanical shaker for 15 min. Simultaneously, a blank run was carried out under similar conditions. The absorbance of the organic layer was then measured against the reagent blank at 420 nm.

Preparation of the Calibration Graph

A calibration graph was constructed by plotting concentration of ethoxide solution against absorbance and was found to obey Beer's law in the range 100–1700 μg ml^{-1}. The average molar absorptivity at 420 nm was found to be 178.6 l mol^{-1} cm^{-1} and the standard error of the mean (average of three determinations) varied from 0.033 to 0.289% (Table 2).

Results and Discussion

Experimental parameters such as pH, reaction time, extraction time and molar ratio were studied and the optimum conditions were established as described above. For the quaternary ammonium sulphonate the absorbance at 420 nm remained constant from pH 8 to 12 whereas for the quaternary ethoxide it increased from pH 6 to 10 and decreased rapidly from pH 11 to 13. Hence we selected pH 10 for the determination. Moreover, the molar ratio between the ethoxide and methyl orange was found to be 1 : 7 compared with 1 : 2 for the sulphonate.

A comparison between titrimetric and spectrophotometric methods for the determination of the ethoxide revealed that the former always gives a higher value, by 0.4–0.5%. This may be due to the consumption of acid by the free sodium ethoxide that is present unreacted during the preparation of the quaternary ethoxide. It was found that such free sodium ethoxide does not interfere in the spectrophotometric method.

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Analytical Study of Bis(aroylhydrazones) of 2,6-Diacetylpyridine: Application to the Spectrophotometric Determination of Antimony in Non-ferrous alloys

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The properties of 2,6-diacetylpyridine bis(2-hydroxybenzoylhydrazone) (DAPBSH) and 2,6-diacetylpyridine bis(benzoylhydrazone) (DAPBBH) are described. The reaction between Sb(III) ions and DAPBBH has been studied spectrophotometrically and the resulting orange - yellow Sb - DAPBBH complex extracted into 3-methylbutan-1-ol ($\varepsilon = 1.13 \times 10^4$ l mol^{-1} cm^{-1} at 405 nm) from pH 0.5 to 5.3. The Sandell sensitivity is 0.0011 µg cm^{-2} in 50 ml of aqueous solution. The method described is highly selective and has been applied satisfactorily to the determination of antimony in non-ferrous alloys.

Keywords: *Bis(aroylhydrazone) reagents; antimony determination; extraction; spectrophotometry; non-ferrous alloys*

The condensation products of pyridinic aldehydes or ketones with hydrazides have proved to be good chromogenic reagents.[1] The chelating behaviour of monoaroylhydrazones of some pyridine carbonyl derivatives towards metal ions has previously been examined and it has been found that salicyloylhydrazones and benzoylhydrazones are excellent organic reagents for the spectrophotometric determination of vanadium(V) and iron(II).[2–5] The introduction of a double hydrazidic chain in pyridine dicarbonyl derivatives gives rise to the formation of reagents with similar characteristics, provided that both chains can rotate freely.[6,7] Among these ligands, the behaviour of 2,6-diacetylpyridine bis(aroylhydrazones) is noteworthy owing to their tendency to produce stereochemistries of high coordination number.[8,9] It seemed appropriate, therefore, to study this family of reagents further.

This paper describes the investigation of the analytical properties of 2,6-diacetylpyridine bis(2-hydroxybenzoylhydrazone) (DAPBSH) and 2,6-diacetylpyridine bis(benzoylhydrazone) (DAPBBH). Using the latter reagent, an extraction - spectrophotometric procedure for the determination of Sb(III) has been developed in which the Sb - DAPBBH complex is extracted into 3-methylbutan-1-ol from strongly acidic media in which other metal - DAPBBH complexes are not completely formed. The proposed method has been applied satisfactorily to the determination of Sb in several standard samples of non-ferrous alloys.

Experimental

Apparatus

A Pye Unicam SP8-200 and a Perkin-Elmer Coleman 575 spectrophotometer with 1-cm optical path cells were used in the ultraviolet and visible region of the spectrum. A Metrohm Herisau Titriskop E-516 pH meter with glass - calomel electrodes was employed for pH measurements and an Apple II desk-top computer was utilised for the evaluation of analytical data.

Reagents

All solutions were prepared with analytical reagent-grade reagents using distilled water, unless stated otherwise.

2,6-Diacetylpyridine bis(2-hydroxybenzoylhydrazone) and bis(benzoylhydrazone) solutions, 0.05% *m/V*. Prepared by dissolving appropriate amounts of the reagents in dimethylformamide (DMF), dimethyl sulphoxide (DMSO) or 3-methylbutan-1-ol. A 0.1% *m/V* solution of DAPBBH in 3-methylbutan-1-ol was also used.

Antimony standard solution, 1.000 g l^{-1}. Prepared by dissolving antimony(III) chloride in 2 N hydrochloric acid and standardising titrimetrically.[10] Working standard solutions were prepared daily from this stock solution.

Procedure for the Direct Determination of Antimony

To 50 ml of sample solution in a separating funnel, containing up to 60 µg of antimony, add 0.5 ml of concentrated perchloric or nitric acid (to adjust the pH to 1) and extract with 5 ml of a 0.1% *m/V* solution of DAPBBH in 3-methylbutan-1-ol by shaking for 3 min. Allow the phases to separate and transfer the upper organic phase, previously dried on anhydrous sodium sulphate, into a 5-ml calibrated flask. Measure the absorbance of the orange - yellow extract at 405 nm against water.

The calibration graph was prepared from standard solutions of Sb(III) treated in the same way.

Procedure for the Indirect Determination of Antimony

To three aliquots of the sample solution, add increasing known amounts of antimony (0, 10 and 20 µg) and apply the extraction procedure as described above. The absorbances are plotted against the concentrations for the three antimony-containing solutions of each sample. The intersection of this curve with the concentration axis is defined by $y = 0$ in the simple linear regression equation $y = ax + b$. When $y = 0$, we find $x = -b/a = C_{\mathrm{Sb}}$, where C_{Sb} is the concentration of antimony in the sample. All parameters in the regression equation curves were calculated by the least-squares method.

Results and Discussion

Analytical Properties of the Reagents

The reagents were synthesised by the general procedure outlined for other related compounds.[4,5] The elemental analyses for C (63.91 and 67.84%), H (4.93 and 5.98%) and N (16.27 and 17.60%) contents agreed with the values calculated from the empirical formulae of DAPBSH ($C_{23}H_{21}N_5O_4$) and DAPBBH ($C_{23}H_{21}N_5O_2$), respectively. The infrared spectra of the reagents, obtained from spectroscopically pure KBr discs, are in good agreement with those obtained by Pelizzi and Pelizzi.[8]

DAPBSH and DAPBBH have a low solubility in several common solvents (Table 1), their solubilities being lower than those of pyridine-2-acetaldehyde 2-hydroxybenzoylhydrazone (PASH)[4] and benzoylhydrazone (PABH),[11] probably owing to the presence of the double hydrazidic chain. The slight solubility difference between the two reagents, as observed in Table 1, shows that the hydroxyl group makes scarcely any contribution to the solubility, presumably as a result of the formation of intramolecular hydrogen bonds. This behaviour is in agreement with the absorption band located at 2800–2500 cm^{-1} in the infrared spectrum of DAPBSH, which can be attributed to the OH stretching vibration.[4,8,9,12]

The spectral characteristics of DAPBSH and DAPBBH in different media are shown in Table 1. The change from water to other less polar solvents resulted in a bathochromic and hyperchromic shift of the main absorption band of DAPBBH. This band is probably due to an $n \rightarrow \pi^*$ transition.[12] The molar absorptivity of this band in chloroform is about twice as large as those of the PASH[4] and PABH[11] bands (λ = 323 nm, $\varepsilon = 1.9 \times 10^4$ l mol^{-1} cm^{-1} and λ = 295 nm, $\varepsilon = 1.9 \times 10^4$ l mol^{-1} cm^{-1}, respectively), as DAPBSH and DAPBBH are the corresponding duplicate chromophores.

Varying the pH affected the spectra of the aqueous solutions of both reagents, both showing bathochromic shifts as the pH increased, which can be assumed to be related to the extension of the π-chromophore.

The Stenström and Goldsmith[13] and Sommer[14] methods were used for the spectrophotometric determination of the ionisation constants of the reagents at 0.1 ionic strength. The average pK_1 values were found to be 2.9 ± 0.1 and 2.7 ± 0.2 for DAPBBH and DAPBSH, respectively. They may be caused by protonation of the pyridine nitrogen atom. The average pK_2 values were found to be 9.6 ± 0.2 and 7.5 ± 0.2 for DAPBBH and DAPBSH, respectively. The first pK may be caused by deprotonation of the CONH group and the second by deprotonation of the hydroxyl group in the benzene ring.[15]

DAPBSH and DAPBBH solutions were stable at pH 4.9, but decomposed in moderately acidic media. However, their resistance to acid hydrolysis was greater than that of related aroylhydrazones, probably because the C=N group is part of a six-membered ring.

Redox reagents (H_2O_2, N_2H_4, ascorbic acid), at concentrations of 0.5% m/V, altered the absorption spectra of the two reagents, DAPBSH being more affected than DAPBBH. Both reagents were more strongly affected by hydrogen peroxide in acidic media and by hydrazine in alkaline solutions.

The reaction features of the reagents with more than 40 species of metal ions were investigated in water - DMF and water - DMSO media at different pH values. A medium containing 50% of organic solvent was chosen because of the slight solubility of both the metal complexes and excess of reagents. The spectral characteristics of the more interesting soluble complexes are shown in Table 2. Most metal complexes were precipitated in water - DMF media or in water - DMSO at pH 4.9 for DAPBBH, whereas for DAPBSH this

Table 1. Spectral characteristics and solubilities of DAPBSH and DAPBBH in some common solvents

		DAPBSH					DAPBBH				
Solvent	D*	λ/nm	$\varepsilon \times 10^{-4}$/ l mol^{-1} cm^{-1}	λ/nm	$\varepsilon \times 10^{-4}$/ l mol^{-1} cm^{-1}	Solubility/ g l^{-1}	λ/nm	$\varepsilon \times 10^{-4}$/ l mol^{-1} cm^{-1}	λ/nm	$\varepsilon \times 10^{-4}$/ l mol^{-1} cm^{-1}	Solubility/ g l^{-1}
Chloroform	4.8	320	3.5	—	—	N.d.†	312	3.9	—	—	0.2
4-Methylpentan-2-one . .	13.1	335	2.5	—	—	0.2	335	1.6	—	—	N.d.
3-Methylbutan-1-ol . .	14.7	320	3.2	290	2.5	1.0	340	2.5	315	2.8	0.9
Ethanol	24.3	320	4.4	280‡	2.8	0.2	335	3.8	305‡	2.9	0.1
Dimethylformamide . .	36.7	305	2.6	280	2.6	1.9	345	2.9	305‡	2.2	1.1
Dimethyl sulphoxide . .	46.7	320	3.4	290	3.0	3.2	335	2.8	305‡	2.2	4.4
Water	78.5	320	2.7	280	2.6	0.3	330	2.1	300	2.4	0.6
Water (pH 3)		300	1.0	265	1.2	—	315‡	1.4	290	1.5	—
Water (pH 4.9)§		310	1.5	275	1.6	—	315‡	2.0	290	2.4	—
Water (pH 9.2)		332	2.8	275	3.2	—	350	2.2	295	3.0	—
Water (pH 9.8)		335	2.2	280	4.1	—	360	1.8	295	3.5	—

* D, Dielectric constant at 25 °C, except for $CHCl_3$ at 20 °C.
† N.d., Not determined.
‡ Shoulder.
§ A shoulder is also observed at 330 nm for DAPBSH.

Table 2. Spectral characteristics of DAPBSH and DAPBBH reactions with metal ions

	DAPBSH*		DAPBBH†	
Metal ion	$\lambda_{max.}$/nm	$\varepsilon \times 10^{-4}$/ l mol^{-1} cm^{-1}	$\lambda_{max.}$/nm	$\varepsilon \times 10^{-4}$/ l mol^{-1} cm^{-1}
Bi(III)	412	34.1	380	18.6
Ca(II)	412	6.6	—	—
Cd(II)	410	12.4	385	14.2
Co(II)	410	4.2	403	5.6
Cu(II)	422	5.6	—	—
Fe(II)	420	7.2	430	1.3
Fe(III)	420	6.3	415	10.4
Hg(I)	410	10.4	400	9.8
Hg(II)	408	2.6	—	—
La(III)	408	12.3	—	—
Ni(II)	407	4.3	—	—
Pb(II)	407	15.8	403	8.2
Mn(II)	—	—	400	7.2
Sb(III)	—	—	403	1.6
Th(IV)	—	—	385	8.7

* In water - DMF (1 + 1) (pH 4.7).
† In water - dimethyl sulphoxide (1 + 1) (pH 9.2).

occurred in water - DMSO media or in water - DMF at pH 9.2. Most of the metal chelates formed by both reagents were decomposed more or less readily as the hydrogen ion concentration was increased. However, the antimony chelate was stabilised by extraction into 3-methylbutan-1-ol, the sensitivity increasing if DAPBBH was used.

Study of the Sb(III) - DAPBBH System

Extraction of the antimony chelate

When a solution of DAPBBH in an organic solvent was shaken with an acidic solution of antimony(III), an orange - yellow complex was formed immediately in the organic phase. 3-Methylbutan-1-ol proved to be the best organic solvent as the resulting complex was extracted completely and with high stability. Antimony(V) did not form any extractable complex with DAPBBH. The absorption spectrum of the Sb(III) - DAPBBH chelate in 3-methylbutan-1-ol is shown in Fig. 1(*a*). This chelate was stable for at least 1 d and showed two intense absorption bands. The first, at 340 nm, can be assigned to an $n \rightarrow \pi^*$ transition in the ligand molecule, and the other at 405 nm is probably due to a metal to ligand charge transfer.[8,9,12] This latter wavelength was employed for absorbance measurements, as the reagent does not absorb at this wavelength. Maximum constant absorbances were obtained over the pH range 0.5–5.3 [Fig. 1(*b*)]. The addition of 0.2–0.5 ml of concentrated perchloric or nitric acid was sufficient to adjust the extraction pH value in both instances.

An aliquot of 10–50 ml of solution containing 20 µg of antimony(III) was extracted with 5 ml of a 0.02–0.1% *m/V* solution of DAPBBH in 3-methylbutan-1-ol. Extractions were quantitative (extraction efficiency higher than 94%) for a 0.1% reagent concentration, but for a reagent concentration of 0.05%, quantitative extractions were observed for V_{aq}/V_o ratios of up to 6 : 1. Therefore, 5 ml of 0.1% reagent solution was chosen as the concentration of the chelating agent-containing solution to be used. On the other hand, if the extraction was carried out with phase-volume ratios higher than 10, the extraction efficiency was less than 87%.

The extraction was complete after a shaking time of 30 s for phase-volume ratios from 2 to 6, using 5 ml of 0.05–0.1% reagent solution. For phase-volume ratios between 6 and 10, a shaking time of 2–5 min was required to achieve complete extraction if a 0.1% reagent solution was used. Therefore, a shaking time of 3 min was selected for carrying out extraction with a phase-volume ratio of 10.

The ionic strength (sodium perchlorate, nitrate or sulphate) in the aqueous phase (I = 0.1) did not affect the absorbance of the extracted complex. However, if sodium chloride was used, an ionic strength of up to 0.2 could be tolerated.

The stoicheiometry of the antimony chelate was determined by Irving and Pierce's method[16] using the experimental data obtained from the extraction system. Two complex species were found with formulae SbL and SbL_2.

Extraction - spectrophotometric determination of antimony

Under the optimum conditions described above, Beer's law was obeyed between 1 and 12 µg ml^{-1} of Sb(III) in the organic phase (5 ml) at 405 nm. The optimum concentration range, evaluated by Ringbom's method, was 1.5–8 µg ml^{-1} of antimony.

The orange - yellow complex gave a molar absorptivity of 1.13×10^4 l mol^{-1} cm^{-1} at 405 nm in 3-methylbutan-1-ol, the Sandell sensitivity (in 50 ml of aqueous phase) being 0.0011 µg cm^{-2}. The mean values for eleven samples each containing 20 µg of antimony gave a relative error for the method of ±0.51%.

Antimony (20 µg per 50 ml of aqueous phase) was determined in the presence of various amounts of foreign ions. Cations and anions were added up to a maximum of 0.2 g. The tolerance limits for several ionic species were substantially increased by the use of masking reactions. Table 3 shows the effects of foreign ions on the extraction - spectrophotometric determination of Sb using DAPBBH.

Applications

To test the reliability of the proposed method, it was applied to the determination of antimony in non-ferrous alloys from the Bureau of Analysed Samples Ltd. and British Chemical Standards. Leaded gunmetal, phosphor bronze and white metal were dissolved in a mixture of concentrated hydrochloric and nitric acids and water (1 + 2 + 2, *V/V*)[17] and duralumin was dissolved in 30 ml of 20% *m/V* sodium hydroxide solution and then treated with 50 ml of hydrochloric acid (1 + 1) and a few drops of hydrogen peroxide.[18]

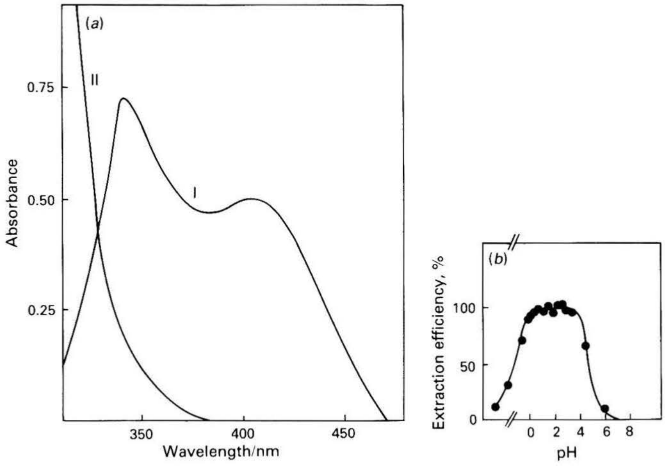

Fig. 1. (*a*) Absorption spectra of (I) Sb(III) - DAPBBH complex (3.29×10^{-5} M) and (II) reagent; (*b*) extraction efficiency *versus* pH of the antimony complex

Table 3. Interferences of foreign ions in the extraction determination of Sb with DAPBBH

Interfering species		Tolerated level (ion: Sb mass ratio)
Without masking reaction	With masking reaction*	
Ba, Sr, Mg, citrate, PO_4^{3-}, Br^-, SO_4^{2-}, CO_3^{2-}, CN^-, N_2H_4, NH_2OH, chloroacetate, Al^{3+}, Cl^-, acetate, NO_3^-, SO_3^{2-}, ClO_4^-, alkali metals	Ca, Be, Pb, Th(IV) (SO_4^{2-}); tartrate, oxalate, PO_4^{3-} (Ba^{2+}); Tl(I) (Cl^-); I^- (oxidation to I_2); NO_2^- (NH_4^+); ClO_3^- (H^+); $S_2O_8^{2-}$ (reduction to SO_4^{2-}); Hg(II) (CN^-)	10 000
Ni, La(III), Cr(III), Th(IV), As(V), $B_4O_7^{2-}$	Co (CN^-); Fe(III) (PO_4^{3-}); Fe(II) (oxidation to Fe^{3+}); BrO_3^- (reduction to Br_2); $S_2O_3^{2-}$ (H^+)	5 000
Ca, tartrate, oxalate, I^-, H_2O_2, Cu(II), Hg(II), Tl(I), Se(IV)	Au(III), Pd(II), Ag(I) (CN^-); Ti(IV), Zr(IV) (PO_4^{3-}); F^- (Al^{3+}); Hg(II) (EDTA)	1 000
SCN^-, EDTA, IO_3^-, As(III), Be, SiO_3^{2-}		500
F^-, NO_2^-, ClO_3^-, Mn(II), Nd(III), Pr(III)		250
Ag(I), IO_4^-, Co(II), Sm(III), Yb(III), Er(III)	S^{2-} (HNO_3); Bi(III) (EDTA)	100
Zn(II), Mo(VI)		50
Cd(II), Sn(II), W(VI)	Ce(IV) (EDTA)	25
Ce(IV), Zr(IV), Au(III), Fe(III)		10
Ti(IV), V(V)		2.5
Fe(II), Bi(III), U(VI), $S_2O_3^{2-}$, $S_2O_8^{2-}$, BrO_3^-, S^{2-}		1

* Masking reagent used is shown in parentheses. If a precipitate appears in the organic phase, centrifuge or filter before measuring the absorbance.

Table 4. Analysis of non-ferrous alloys

Sample	Mass/g	Antimony content, %		
		Found	Average found	Certified
White metal* (BAS No. 8e)	0.7601	9.49 ± 0.05	9.56 ± 0.09	9.5
	0.3754	9.63 ± 0.07		
Leaded gunmetal (BAS No. 77a)	0.4065	0.234 ± 0.008	0.241 ± 0.010	0.24
	0.8435	0.246 ± 0.010		
	0.7239	0.245 ± 0.007		
Phosphor bronze† (BAS No. 7a)	0.8690	0.064 ± 0.001	0.070 ± 0.002	0.07
	0.8698	0.077 ± 0.002		
Duralumin (BCS No. 216/2)	0.8255‡	0.033 ± 0.003	0.028 ± 0.008	0.03
	0.8333‡	0.028 ± 0.001		
	0.7341‡	0.026 ± 0.002		
	1.1831‡	0.027 ± 0.003		
	0.8928‡	0.032 ± 0.001		
	1.1831†	0.027 ± 0.007		

* Direct method.
† In the presence of EDTA.
‡ In the presence of phosphate.

The standard additions method was employed in all instances (all regression curves were almost linear, the correlation coefficients being 0.993 or higher), except for white metal. In order to prevent interferences due to foreign ions, 50 mg of phosphate or 25 mg of EDTA were added to solutions of duralumin or phosphor bronze. The results obtained for the triplicate analysis of different aliquots of different sample solutions are shown in Table 4. These data demonstrate the precision and reliability of the proposed method for the determination of antimony in the analysed samples.

Conclusion

Other methods are available for the photometric determination of trace amounts of antimony, but many of them lack high sensitivity and selectivity. This paper describes a study of the optimum conditions for a sensitive and selective spectrophotometric procedure for the determination of antimony in non-ferrous alloys. The method is free from interferences because most metallic chelates of DAPBBH are not extracted into 3-methylbutan-1-ol from strongly acidic media. The good results obtained by using masking reactions are due to the fact that the precipitates formed usually remain in the lower aqueous phase. On the other hand, the DAPBBH method may be applied to the determination of Sb at the micrograms to nanograms per millilitre level, provided optimum higher phase-volume ratios are taken, thereafter applying the standard additions method.[4]

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Extraction and Spectrophotometric Determination of Titanium(IV)

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An extraction - spectrophotometric procedure for the determination of titanium(IV), based on the formation of a Ti - *N*-4-chlorophenyl-3,4,5-trimethoxycinnamohydroxamic acid (Ti - PTCHA) chelate that is extractable into chloroform from strongly acidic media, is described. The complex exhibits maximum absorbance at 380 nm with a molar absorptivity of 1.7×10^4 l mol^{-1} cm^{-1}. A comparison of the sensitivity of several synthesised hydroxamic acids with an extraction - photometric determination of titanium(IV) has been made. Various parameters for optimising the extraction conditions including the effect of thiocyanate are discussed.

Keywords: *Titanium(IV) determination; extraction;* N*-4-chlorophenyl-3,4,5-trimethoxycinnamohydroxamic acid; spectrophotometry*

Several hydroxamic acids have been reported for the extraction - photometric determination of various metal ions,[1-4] but only a few on them have been studied as reagents for the quantitative determination of titanium(IV). Titanium forms a yellow complex with hydroxamic acids, which can be extracted into organic solvents at higher acidities. It has been observed that changes in the substitution of the *N*- and *C*- phenyl ring of hydroxamic acids alter the sensitivity and selectivity of the reagent towards metal ions. *N*-Phenylbenzohydroxamic acid has previously been used for the determination of titanium.[5,6] The complexes formed have maximum absorbances at 380 and 420 nm, with molar absorptivities of 6.7×10^3 and 3.8×10^3 l mol^{-1} cm^{-1}, respectively. However, the addition of thiocyanate has been found to enhance the sensitivity of the method because of the formation of the ternary complex. The sensitivity of the determination has also been increased by the use of *N*-phenylcinnamohydroxamic acids owing to increased conjugation in the *C*-phenyl ring.[7-9] However, interferences from diverse ions have been found to be the same. *N*-4-Chlorophenyl-3,4,5-trimethoxycinnamohydroxamic acid has been reported for the extraction and spectrophotometric determination of niobium(V).[10] In this work five new hydroxamic acids have been synthesised and their sensitivity and selectivity towards the extraction - photometric determination of titanium have been explored. Among the acids studied, *N*-4-chlorophenyl-3,4,5-trimethoxycinnamohydroxamic acid (PTCHA) was found to be the most sensitive reagent for titanium. The effects of thiocyanate, acidity, reagent concentration and interferents on the extraction - spectrophotometric determination of titanium are also reported.

Experimental

Chemicals

All of the chemicals used were of AnalaR grade obtained from BDH Chemicals. The preparation of the hydroxamic acids was based on the procedure described elsewhere[11] and the acids were crystallised from benzene (**Caution**—Benzene is a highly toxic substance and the appropriate precautions should be taken). A 0.1% *m*/*V* solution of the reagent in chloroform was used for extraction purposes.

The titanium solution was prepared by heating 0.20 g of TiO_2 in a Pyrex flask with 8.0 g of ammonium sulphate and 25 ml of concentrated sulphuric acid for 4 h. After cooling, the resulting solution was transferred into a 250-ml calibrated flask containing 150 ml of water. The Pyrex flask was washed with 5% sulphuric acid, the washings were transferred into the calibrated flask and the contents diluted with 5% sulphuric acid. The final concentration (1.46×10^{-2} M) was determined tritrimetrically[12] and colorimetrically.[5,6] The thiocyanate solution (6 M) was prepared by dissolving the required amount of ammonium thiocyanate in distilled water and standardising by Volhard's method.[13]

Spectral measurements were made on a VSU 2-P spectrophotometer with 1-cm cells.

Procedure

Transfer an aliquot (1 ml) of Ti(IV) solution containing 28 µg of the metal into a separating funnel and add 4 ml of distilled water and 20 ml of 11.25 M HCl so as to obtain an aqueous phase of 9 M HCl in a total volume of 25 ml. Add 8 ml of 0.1% reagent in chloroform and shake the contents for 10 min. Collect the organic layer in a 25-ml calibrated flask after drying over anhydrous sodium sulphate. Repeat the extraction with 2 ml of the reagent to ensure complete recovery of the titanium. Collect the extracts in the same flask and dilute to the mark with chloroform. Measure the absorbance of the yellow complex at 380 nm against a reagent blank prepared similarly.

To study the ternary complex of titanium - hydroxamate with thiocyanate, add 1 ml of 6 M ammonium thiocyanate solution to the mixture, prior to the acidity adjustment, and extract the complex as described above.

Determination of Titanium in Standard Samples

The sample solution was prepared by dissolving the standard steel sample in hydrofluoric acid and evaporated to dryness. The sample was then allowed to stand on a steam-bath for about 1 h until acid fumes were no longer evolved. The residue was heated with 10 ml of concentrated sulphuric acid and diluted to 500 ml with 4% sodium oxalate solution. To ensure the complete removal of hydrofluoric acid from the sample a small portion of the solution was tested for fluoride.[14] Next, an aliquot was taken and 1.0 ml of 0.1% tin(II) chloride solution was added to reduce iron(III), vanadium(IV), etc. The titanium content was determined according to the procedure described above.

Results and Discussion

Absorption Spectra and Beer's Law

The spectral characteristics of the titanium - hydroxamate complexes are summarised in Table 1. A solution of PTCHA in chloroform extracted Ti(IV) at an acidity of 8–11 M HCl. The extracted complex exhibits maximum absorbance at 380 nm with a molar absorptivity of 1.7×10^4 l mol^{-1} cm^{-1}; the reagent has an absorption maximum at 326 nm in ethanol.

Table 1. Spectral characteristics of Ti - *N*-arylhdroxamic acid complexes in chloroform. [Ti] = 28 μg per 25 ml; reagent, 10 ml (0.1% in chloroform)

Compound No.	Hydroxamic acid	λ_{max}/nm	Molar absorptivity/ l mol^{-1} cm^{-1}
1	*N*-4-Chlorophenyl-3,4,5-trimethoxycinnamo-	380	1.7×10^4
2	*N*-4-Chlorophenyl-4-butoxybenzo-	410	6.5×10^3
3	*N*-Phenyl-4-butoxybenzo-	410	6.3×10^3
4	*N*-4-Chlorophenyl-4-chlorophenoxyisobutyro-	410	1.4×10^3
5	*N*-Phenyl-4-chlorophenoxy-isobutyro-	410	1.3×10^3

Table 2. Effect of the concentration of HCl on the extraction of titanium with *N*-4-chlorophenyl-3,4,5-trimethoxycinnamohydroxamic acid. [Ti] = 28 μg per 25 ml; $\lambda_{max.}$ = 380 nm

HCl concentration/ M	Extraction, %	Molar absorptivity/ l mol^{-1} cm^{-1}
2	45	0.4×10^4
4	51	0.8×10^4
6	61	1.0×10^4
7	86	1.4×10^4
8	100	1.7×10^4
9	100	1.7×10^4
10*	100	1.7×10^4
11*	100	1.7×10^4

* The extract becomes turbid at this concentration.

Table 3. Effect of reagent concentration on the extraction of titanium. Reagent, 0.1% PTCHA in chloroform; [Ti] = 28 μg per 25 ml; $\lambda_{max.}$ = 380 nm; and [HCl] = 9 M

Volume of reagent/ml	Absorbance	Molar absorptivity/ l mol^{-1} cm^{-1}
0.1	0.050	0.2×10^4
0.2	0.095	0.4×10^4
0.4	0.350	1.0×10^4
1.0	0.350	1.5×10^4
2.0	0.400	1.7×10^4
4.0	0.400	1.7×10^4
10.0	0.400	1.7×10^4
15.0	0.400	1.7×10^4
20.0	0.395	1.7×10^4

Table 4. Effect of diverse ions. [Ti] = 28 μg per 25 ml

Foreign ion	Added as	Tolerance limit/ mg
Ag^+	$AgNO_3$	25
Cd^{2+}	$CdSO_4$	30
Pb^{2+}	$Pb(NO_3)_2$	30
Be^{2+}	$BeSO_4$	30
Mg^{2+}	$MgSO_4$	30
Ca^{2+}	$Ca(NO_3)_2$	30
Ba^{2+}	$BaCl_2$	25
Sn^{2+}	$SnCl_2$	25
Co^{2+}	$CoCl_2$	25
Cu^{2+}	$CuSO_4$	30
Hg^{2+}	$HgCl_2$	25
Ni^{2+}	$NiCl_2$	20
Zn^{2+}	$ZnSO_4$	35
Mn^{2+}	$MnCl_2$	20
Cr^{3+}	$CrCl_3$	20*
Al^{3+}	$AlCl_3$	35
V^{5+}	NH_3VO_3	25*
$Mo_7O_{24}{}^{6-}$	$(NH_4)_6Mo_7O_{24}$	10*
Zr^{4+}	$Zr(NO_3)_4$	5*
Cl^-	NaCl	40
Br^-	NaBr	15
I^-	NaI	20
CH_3COO^-	CH_3COONa	40
Citrate^{3-}	Citric acid	40
$SO_4{}^{2-}$	Na_2SO_4	40

* Masking agents used.

Table 5. Determination of titanium in standard samples

No.	Sample (BCS)	Titanium content, %: Certified value	Obtained by present method*	Obtained by AAS
243	Ferrotitanium (Ti,Al,C,Cu,Si,Mn)	40.00	39.80	40.10
236	Cast iron (Si,S,P,Mn,Ti)	0.102	0.10	0.10
182	Silicon - aluminium alloy (Se,Fe,Cu,Mn,Mg,Ti, Zn, Ni)	0.210	0.20	0.21

* The values are averages of eight determinations.

Other hydroxamic acid - titanium complexes have absorbance maxima between 400 and 420 nm and hence all the measurements were made at 410 nm and the absorbance was found to be reproducible in all instances.

Beer's law is obeyed in the range 0.1–3.3 p.p.m. of titanium as the Ti - PTCHA complex at 380 nm.

Effect of Acidity

There was no noticeable colour observed in the chloroform extract for up to 5 M HCl but the complex was found to be extractable from 6 M HCl with the formation of a yellow coloration. The extraction increased with increase in the molarity of HCl, reaching a maximum at 9.5 M HCl (Table 2). At higher acidities, the chloroform extract was turbid, and difficult to dry over sodium sulphate. The optimum molarity was taken as 9.0 M HCl and all subsequent studies were carried out at this molarity.

Effect of Reagent and Time

Titanium (28 μg) was extracted using varying concentrations of reagent. It can be seen from the results presented in Table 3 that 2 ml of 0.1% reagent solution was satisfactory for the complete extraction of titanium. A slight excess of the reagent was also used without any difficulty, but a large excess of reagent increased the blank absorbance.

A shaking time of 5 min was adequate for quantitative extraction of the titanium and the chloroform extracts of the complex were stable for 2 d.

Effect of Ammonium Thiocyanate

The sensitivity of the method was enhanced by the use of thiocyanate for the extraction of titanium with hydroxamic acid as the Ti - PTCHA - SCN ternary complex. The results indicated that a maximum volume of 1 ml of ammonium thiocyanate (6 M) was sufficient for ternary complex formation and the molar absorptivity of the Ti - PTCHA - SCN complex was found to be 2.7×10^4 l mol^{-1} cm^{-1} at 380 nm.

Stoicheiometry

The composition of the extracted species was studied by Job's continuous variations method[15] and the slope ratio method.[16] Using either method the metal to ligand ratio was found to be

1:2 for the binary (Ti - PTCHA) complex and 1:2:1 for the ternary (Ti - PTCHA - SCN) complex. The reaction mechanism is as follows:

$$TiOCl_2 + 2HA \xrightarrow{HCl} (TiOClA_2)^- + 2H^+ + Cl^-$$

$$(TiOClA_2)^- + SCN^- \xrightarrow{HCl} (TiOA_2SCN)^-H^+ + Cl^-$$

Effect of Diverse Ions

Titanium (28 μg per 25 ml) was determined in the presence of a large number of diverse ions (Table 4). Moderate amounts of various metal ions and anions commonly associated with titanium were tolerated. However chromium (>10 p.p.m.), vanadium (>1 p.p.m.), molybdenum (>1 p.p.m.) and zirconium (>6 p.p.m.) interfered in this method. The interference from vanadium was overcome by adding $FeSO_4$ and that due to molybdenum was removed by precipitation with sulphide prior to extraction. There was some interference from Nb (>5 p.p.m.) but this was removed by precipitating the excess of Nb with PTCHA in 1 N H_2SO_4, keeping the Ti in solution with EDTA and H_2O_2. Moderate amounts of zirconium and chromium were tolerated in the presence of tin(II) chloride. Fluoride (>2 p.p.m.) was also found to interfere.

The results for the determination of titanium in steel are given in Table 5.

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Spectrophotometric Determination of Boron

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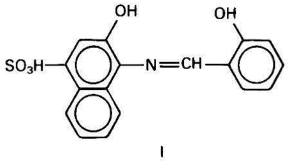
The azomethine derivatives 8-hydroxy-1-(2-hydroxy-1-naphthylbenzylideneamino)naphthalene-3,6-disulphonic acid (HSNHB) and 8-hydroxy-1-(2-hydroxy-1-naphthylmethyleneamino)naphthalene-3,6-disulphonic acid (HSNHN) were synthesised. HSNHB gives no colour reaction with boric acid, but HSNHN reacts with boric acid and produces a compound with maximum absorbance at 445 nm (pH 4) in a 30% *V*/*V* methanol - water solution. Beer's law is obyed between 0.25 and 2 μg ml^{-1} of boron. The molar absorptivity at 445 nm is 8700 l mol^{-1} cm^{-1}. Interferences from Fe^{3+}, Al^{3+} and Cu^{2+} are avoided by adding EDTA. This method has been applied to the determination of boron in natural waters and vegetable matter with good accuracy.

Keywords: *Boron determination; complex formation; spectrophotometry; natural waters*

The determination of boron at trace levels is very important in areas as varied as the electronic, preserved foods, agricultural and nuclear industries.[1] Nevertheless, the determination of boron at the p.p.m. to p.p.b. level continues to present some difficulty. Although many of the available methods are sensitive,[2] they lack accuracy or reproducibility[3] and require very specific experimental conditions for application to real samples.[4–6]

The reactions and properties of azomethine[7,8] have been studied in detail and in 1976 it was adopted as the standard method for the determination of boron by the US Department of Agriculture.[9] To date, only new positions for sulphonic groups have been studied (azomethine H and K), but not the possibility of using new derivatives that maintain the azomethine structure but modify the condensed groups and therefore create better reaction conditions.

For this purpose, **3-hydroxy-4-(2-hydroxybenzylideneamino) naphthalene-1-sulphonic acid (HSNHB) (I)** Ch.S. 5 Nov 85 and 8-hydroxy-1-(2-hydroxy-1-naphthylmethyleneamino)naphthalene-3,6-disulphonic acid (HSNHN) (**II**) have been synthesised by nucleophilic condensation of the corresponding aldehyde and amine.[12] The reaction of these reagents with boron has been studied.



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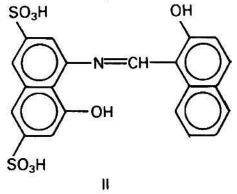

II

Experimental

Apparatus

A Pye Unicam SP 8-100 UV - visible spectrophotometer, a Kotterman mechanical shaker, a Gebruder-Hoake thermostatic bath and PTFE- or plasticware, to avoid boron contamination from glass, were used.

Reagents and Solutions

Standard boron solution, 1000 μg ml^{-1}. Dissolve 5.715 g of pure boric acid (Merck) in a 1-l calibrated flask and dilute to volume with doubly distilled water. Mix thoroughly and transfer the solution into a plastic bottle. Dilute solutions are prepared just before use.

HSNHB solution, 3% *m*/*V*. Dissolve 3 g of HSNHB in 100 ml of doubly distilled water.

HSNHN solution, 3% *m*/*V*. Dissolve 3 g of HSNHN in 100 ml of methanol - water (70 + 30 *V*/*V*).

The above reagents were synthesised as previously described[12] and purified by successive recrystallisations from ethanol - water. Characterisation and purity were verified by elemental analysis, IR spectroscopy, UV - visible spectrophotometry and TLC.

Results and Discussion

Reactions with Boric Acid

Neither a spectrophotometric nor a fluorimetric reaction with the reagent HSNHB was observed. This result is in agreement with the theories of Poluektov and Nikonova.[11] In contrast, the HSNHN reagent changed from yellow to orange in the solutions containing boron. This difference was much more intense in neutral or slightly acidic media. The compound formed had a maximum absorbance at 445 nm but did not fluoresce (Fig. 1).

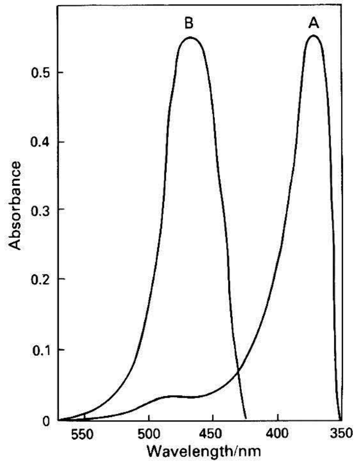

Fig. 1. Absorption spectrum of A, HSNHN (500 μg ml^{-1}) and B, of its complex with boron (1.5 μg ml^{-1}) in methanol - water solution (70 + 30)

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Study of the Optimun Reaction Conditions

The effects of various parameters on the absorbance of the boron - reagent complex were studied, and the reaction conditions were optimised.

Procedure

Boron solutions containing 1.5 µg ml^{-1} of boron were prepared from a standard boron solution. Reagent, in an amount at least six-fold in excess of boron, was added and diluted to 25 ml with pH 4 buffer solution. This was kept in the dark for 3 h and the absorbance was measured at 445 nm against a blank boron-free reagent prepared in the same way.

Effect of pH

Fig. 2 shows the variation of the absorbance at 445 nm with change in pH. The maximum value was obtained at pH 4 and remained constant at pH values between 3.5 and 4.5.

Effect of standing time and temperature

The necessary time for colour development under acidic conditions (pH 4) and with a fixed excess of reagent for the spectrophotometric determinations was studied and the results are shown in Table 1. If the solutions were exposed to light, a rapid increase in the absorbance was observed for up to 1 h; a precipitate then formed, which made measurement of the absorbance impossible. If the solutions were kept in the dark, maximum absorbance was attained after 2 h. This value remained constant for 48 h.

In an attempt to increase the rate of reaction the reaction temperature was increased and the results are shown in Fig. 3. Solutions and blanks were heated to 40, 60 and 80 °C for different periods of time. When the solution was heated, a decrease in absorbance was initially observed—the higher the temperature the greater the decrease with a subsequent slow increase until the original value was attained. This observation can be explained by considering the reaction mechanism of azomethines established by Koulgber,[10] in which boron acts as a catalyst in the reaction between hydrolysed reagent components.

Concentration of reagent

The amount of reagent necessary to obtain the maximum absorbance for a known boron concentration was studied. Solutions were prepared containing boric acid and reagent in different molar ratios, at constant pH and volume of methanol. Absorbance values obtained at 445 nm are shown in Table 2, where it can be seen that it is necessary to add a six-fold excess of reagent in order to reach the maximum absorbance value.

Order of addition of the reagents

The order of addition of the reagents had no effect on the reproducibility of the results and the order of addition followed in all determinations was boron solution, buffer solution and reagent solution.

Beer's law verification

Beer's law was obeyed over the range 0.2–2.5 µg ml^{-1} of boron. The equation of the calibration graph was $A = 0.8048x - 0.025$, where A is the measured absorbance and x the concentration of boron in solution in µg ml^{-1}. The regression coefficient was 0.993, the molar absorptivity 8.7×10^3 l mol^{-1} cm^{-1} and the Sandell sensitvity 0.012 µg cm^{-2} of boron.

Influence of foreign ions

The variation of the absorbance at 445 nm in the presence of foreign ions is shown in Tables 3 and 4. The interference limit was considered as the concentration of interferent that produced an error higher than 2%. Li^+, Na^+, K^+, Ca^{2+}, Ba^{2+}, AsO_2^-, Pb^{2+}, Sn^{4+}, Sr^{2+}, SO_4^{2-}, Cl^- and PO_4^{3-} do not interfere in the determination at high concentrations; Cr^{3+}, Mo^{4+}, Zr^{4+}, Co^{2+}, Ni^{2+} and Zn^{2+} do not interfere at double the concentration of boron; Cu^{2+} and Fe^{3+} give yellowish precipitates; V(V) and Al^{3+} interfere owing to the formation of a yellow compound; and F^- interferes by masking boron.

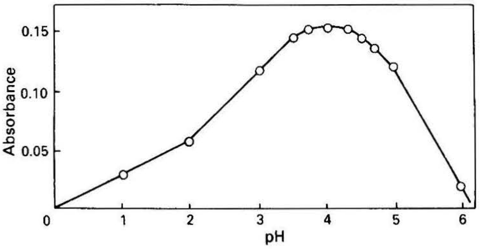

Fig. 2. Variation of absorbance with pH for the boron - HSNHN complex

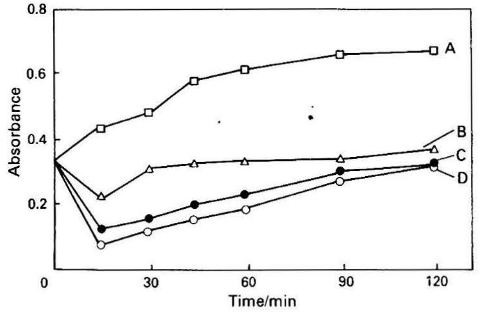

Fig. 3. Variation of absorbance with temperature for boron - HSNHN complex. Temperature: A, 20; B, 40; C, 60; and D, 80 °C

Table 1. Effect of standing time of reaction on absorbance; boron concentration; 1.5 µg ml^{-1}; λ = 445 nm

	Absorbance	
Time	Darkness	Light
0	0.160	0.163
10	0.214	0.154
20	0.308	0.234
60	0.566	—
120	0.750	—
180	0.750	—
600	0.748	—
1440	0.751	—
2880	0.746	—

Table 2. Effect of reagent concentration on the reaction

Ratio of boron to reagent, *m*/*m*	1 : 1	1 : 2	1 : 3	1 : 4	1 : 5	1 : 6	1 : 7
Absorbance (λ = 445 nm)	0.225	0.523	0.727	0.997	1.223	1.293	1.296

Table 3. Influence of foreign ions on the absorbance. Concentration of boron, 2 μg ml^{-1}

Interferent	Amount added/μg	Molar ratio, boron: foreign ion	Variation, %
Cr^{3+}, Mo^{3+}, Zr^{4+}	400	1:2	−1.63
Co^{2+}	800	1:8	1.25
AsO_2^-, Sn^{2+}, Ni^{2+}, Zn^{2+} ..	1000	1:10	0.50
Na^+, K^+, Ba^{2+}, Pb^{2+}	2000	1:20	0.03
Li^+	5000	1:50	0.05
Sr^{2+}	6000	1:60	0.04
SO_4^{2-}, Cl^-, PO_4^{3-}	10000	1:100	0.01

Table 4. Study of interferents in the determination of boron. Concentration of boron, 2 μg ml^{-1}

Interferent	Interference level/μg ml^{-1}	Molar ratio, boron: foreign ion	Absorbance	Variation, %
V(V)	0.4	1:0.2	1.501	0.54
	2	1:1	1.613	+8.03
	4	1:2	1.702	+14.80
	8	1:4	1.792	+20.00
Cu^{2+}	4	1:2	1.513	+1.32
	8	1:4	1.539	+3.11
	12	1:6	Precipitate	—
Fe^{3+}	2	1:1	1.418	−5.02
	8	1:4	1.402	−6.07
	12	1:6	1.386 (turbid)	−7.15
	40	1:20	Precipitate	—
Al^{3+}	8	1:4	1.473	−1.36
	12	1:6	1.472	−1.40
	20	1:10	1.471	−1.46
	40	1:20	1.443	−3.38
	80	1:40	1.449	−2.97
F^-	2	1:1	1.437	−3.75
	4	1:2	1.430	−4.22
	8	1:4	1.397	−6.43

Table 5. Determination of boron in vegetable matter with HSNHN

Plant	Mean* value/μg	CIIAF value/μg	Standard deviation/μg	Variation, %
Rubber	50.23	55.86	2.41	7.5
Palm	13.28	14.51	5.1	6.26
Gum	33.55	34.12	2.23	1.26
Grape	45.16	49.28	1.39	6.17
Orange	39.07	40.04	2.38	2.10
Olive	17.60	17.91	1.76	1.24
Peach	34.61	37.33	1.05	5.35
Croton	25.47	25.27	3.89	0.56
Maize	24.30	23.22	4.30	3.21
Apple	35.00	32.87	2.42	4.44
Apple (golden)	26.60	28.35	2.84	4.50
Cotton	26.40	24.87	3.15	4.22

* Results of ten determinations.

Table 6. Results of analysis of water samples

Sample No.	Source	Mean value/ μg ml^{-1}	Standard deviation/μg ml^{-1} (n = 6)	Relative standard deviation, %
1	Well (A)	0.70	0.009	1.3
2	Well (B)	0.71	0.014	2.0
3	Well (C)	0.68	0.015	2.2
4	Overflow (A)	1.02	0.017	1.7
5	Overflow (B)	0.99	0.024	2.4
6	Overflow (C)	1.20	0.016	1.3
7	River Duero (1)	0.20	0.019	9.5
8	River Duero (2)	0.25	0.006	2.4
9	River Duero (3)	0.18	0.016	8.9
10	Conduction, urban drinking 1	0.09	0.012	13.3
11	Conduction, urban drinking 2	0.11	0.009	8.2
12	Conduction, urban drinking 3	0.10	0.010	10.0
13	Irrigation R1	0.16	0.014	8.8
14	Irrigation R2	0.09	0.020	22.0
15	Irrigation R3	0.20	0.031	15.5

The use of a 1.5 molar excess of EDTA solution eliminated the interferences produced by Fe^{3+}, Cu^{2+} and Al^{3+} but not those produced by F^- and V(V). The F^- interference can be eliminated by adding $ZrCl_4$ to the solution.

HSNHN was used for the determination of boron in vegetable matter (Table 5) and natural waters (Table 6). Values obtained from vegetable matter samples were compared with those given for the same samples by the Nimes Comité Inter-Institutes pour l'Analyse Foliaire (CIIAF).[13] For the determination of boron in waters, the standard additions method was used (2 μg ml^{-1}). The average recovery was 99.3% (average of ten determinations) with a standard deviation of 2%.

When results obtained with the HSNHN reagent in aqueous and organic[12] phases were compared a higher sensitivity after extraction was observed but this makes the method more complicated because of both the extraction and heating steps involved. Further, only V(V) interfered in the aqueous phase so the use of the method described above is suitable for samples, such as vegetable matter and waters, where the sensitivity attained is acceptable.

On the other hand, the presence of NO_3^- does not interfere in the determination of boron with HSNHN in the aqueous phase but does so in the azomethine H method. Therefore, the HSNHN method is particularly convenient when treatment of the sample with nitric acid is necessary (*i.e.*, for vegetable matter).

Conclusion

The described procedure allows the determination of about 2 μg of boron with a Sandell sensitivity of 1.24×10^{-3} μg cm^{-2}. The reaction occurs in the aqueous phase at pH 4 and 60 °C over a period of 45 min. Interferences can be eliminated with EDTA and the method can be applied to the determination of boron in vegetable matter and waters.

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2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol as a Photometric Reagent for the Spectrophotometric Determination of Chromium(III) and/or Chromium(VI) and Its Application to the Sequential Determination of Chromium(III) and Chromium(VI) in Mixtures

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Three simple, rapid and accurate spectrophotometric procedures are described for the individual determination of chromium(III) and chromium(VI) and the sequential determination of chromium(III) and chromium(VI) in mixtures. The first two procedures are based on measuring the absorbance ($\varepsilon = 2.64 \times 10^4$ at 580 nm) of the coloured product as formed by the reaction between chromium(III or VI) and 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP); these two procedures are suitable for the individual determination of chromium(III) or chromium(VI), respectively, in amounts up to about 50 µg per 50 ml when either of them is present in the sample without prior conversion of the valence state of the chromium by additional oxidation or reduction. The third procedure, for the sequential analysis of chromium(VI) and chromium(III), involves the determination of the total amount of chromium with 5-Br-PADAP after the determination of chromium(VI) with diphenylcarbazide [without interference from chromium(III)] and destruction (by boiling) of the excess of diphenylcarbazide and the chromium(III) - diphenylcarbazone complex.

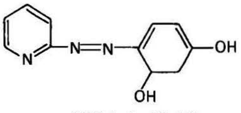
The procedures have been applied to the determination of chromium(VI) and chromium(III) in three water samples containing both chromium(III) and chromium(VI) without the need for any additional separation process, masking or solving of simultaneous equations.

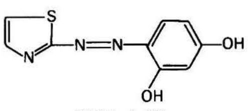
Keywords: *Chromium(III) (and/or chromium VI) determination; sequential spectrophotometry; water analysis; 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol; valence state analysis*

It is well known that chromium occurs widely in the trivalent and hexavalent states in natural and industrial waste waters. Chromium, especially chromium(VI), is harmful to animals and plants and its micro-determination is becoming a subject of great interest. Consequently, there is a need for analytical procedures for the routine micro-determination of chromium.

Some photometric methods for chromium(III or VI) have been published, including those involving diphenylcarbazide,[1,2] the disodium salt of chromotropic acid,[5] xanthene reagent,[4] extraction of perchromic acid,[5] chromium(VI) ion-association complex with Methyl Violet[6] or other triphenylmethane dyes,[7–11] azo based reagents[12–21] and other chelating organic reagents.[21–34] A comparative study of six organic reagents for the determination of chromium(III) has also appeared.[35] With these methods, however, prior to the determination, chromium(III) usually has to be oxidised to chromium(VI). When chromium(III) and chromium(VI) are present together, they are generally separated by precipitation, chromatography, electrophoresis or extraction before either chromium(III) or chromium(VI) is determined spectrophotometrically. Moreover, no one has hitherto suggested a method for determining directly both the individual chromium(III or VI) alone and the total amount of chromium together in waste waters without prior conversion of the valance state of chromium.[36,37] This led us to develop procedures for the individual and successive spectrophotometric determination of chromium(III) and/or chromium(VI).

2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) has been applied to the spectrophotometric determination of many ions, including cadmium(II), cobalt(II), copper(II), iron(II and III), mercury(II), manganese(II), nickel(II), zinc(II), antimony(III), vanadium(IV and V), niobium(V), tantalum(V), uranium(VI),[38] palladium(II), bismuth(III), gallium(III), indium(III), thallium(III), europium(III), zirconium(IV),[39] lead(II)[40] and titanium(IV).[41] In comparing the molecular structures of the following reagents:


PAR (refs. 12–14)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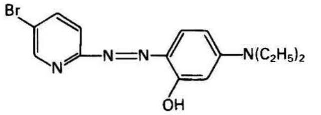
5-Br-PADAP

it might be expected that a colour reaction between chromium(III) and 5-Br-PADAP would also occur and that heating might accelerate the reaction. The results of our preliminary experiments were promising.

In this work, three procedures have been devised to determine chromium(III or VI) individually and to determine sequentially both chromium(III) and chromium(VI) in tap-water or waste water, respectively. The last procedure is a type of multi-component spectrophotometry.[42]

Experimental

Apparatus

All absorbance measurements were made with a Type 721 spectrophotometer (made in China) suitable for measuring absorbance from 360 to 800 nm. A Type S-2 pH meter (made in China) was used for all pH measurements.

Reagents

All reagents must be of analytical-reagent grade unless specified otherwise.

2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) solution, 0.04 *and* 0.02% *m/V*. Dissolve 0.16 mg of

5-Br-PADAP (the systhesis of which was described by Gusev and Schurova[43] and Johnson and Florence[44]) in 400 ml of absolute ethanol to give a 0.04% solution; if necessary, dilute to 800 ml with absolute ethanol (0.02% solution).

Acetate buffer solution, pH 4. Dissolve 41 g of anhydrous sodium acetate in water, add 162 ml of glacial acetic acid and dilute to 500 ml with water.

Diphenylcarbazide solution, 0.25% *m/V in acetone.* Dissolve 0.25 g of the reagent in 100 ml of acetone containing 1 ml of H_2SO_4 (1 + 9). Store in a dark bottle; prepare fresh daily.

2,4-*Dinitrophenol solution, saturated aqueous.*

Chromium(IV) stock solution, 1000 μg ml^{-1}. Dissolve 0.2830 g of $K_2Cr_2O_7$, previously dried at 140 °C, in water and dilute to 1 l in a calibrated flask.

Chromium(VI) standard solution, 10.0 μg ml^{-1} (1.92×10^{-4} M). Dilute a 1.00-ml portion of the chromium(VI) stock solution to 100 ml with water.

Chromium(III) stock solution, 1060 μg ml^{-1}. Dissolve 0.52 g of metallic chromium (purity 99.99%) in 20 ml of concentrated hydrochloric acid plus a small volume of water, with warming to aid solution. Evaporate nearly to dryness, cool and dissolve the residue in water. Transfer the solution into a 500-ml calibrated flask, dilute to the mark with water and mix well, the solution being almost neutral.

Chromium(III) standard solution, 10.6 μg ml^{-1} (2.04×10^{-4} M). Dilute 10.00 ml of the stock solution to 1000 ml with water in a calibrated flask.

Sulphuric acid solution, 0.5 N.

Sodium hydroxide solution, 0.5 N.

Cetyltrimethylammonium bromide (CTMAB) solution, 10^{-2} M. Dissolve 3.64 g of CTMAB in 1 l of aqueous ethanol (2 + 3).

Preliminary Experiments

The results showed the following: (a) the colour reaction between chromium(III or VI) and 5-Br-PADAP occurs on heating the reactants, but chromium(VI) consumes more reagent than chromium(III), because the chromium(VI) is first reduced by 5-Br-PADAP to chromium(III), which then can be reacted with remaining reagent; (b) complexes formed by chromium(III) and chromium(VI) with 5-Br-PADAP have identical absorption spectra; (c) there is no interference from chromium(III) in the determination of chromium(VI) with diphenylcarbazide; and (d) in the sequential spectrophotometric determination of these two ions, the chromium(III) - diphenylcarbazone complex and excess of diphenylcarbazide may be destroyed by boiling the resulting solution, so it would not affect the next determination of chromium(III and VI) with 5-Br-PADAP in the same sample solution.

Procedure

Determination of chromium(III)

By pipette, place a volume of sample solution containing up to 50 μg of chromium(III) into a 50-ml calibrated flask. Add 7 ml of buffer solution and 3 ml of 0.02% 5-Br-PADAP solution, mix, heat the solution in a boiling water-bath for 45 min and cool rapidly to room temperature. Add 1 ml of CTMAB solution and dilute to the mark with water. Measure the absorbance in a 1-cm cell at 580 nm against a reagent blank. Calculate the chromium(III) concentration from a calibration graph prepared by taking aliquots containing known amounts of chromium(III) in the range 0–50 μg per 50 ml of final solution through the procedure.

Determination of chromium(VI)

The determination was carried out as described above, except that a solution containing up to 50 μg of chromium(VI) was used as the sample solution and 0.04% of 5-Br-PADAP as the reagent. The results were calculated by reference to the corresponding calibration graph.

Sequential spectrophotometric determination of chromium (III) and chromium(VI) in mixtures

The procedure involves the following three steps:

Determination of chromium(VI) with diphenylcarbazide in the presence of chromium(III). Place 5 ml of sample solution containing not more than 50 μg each of chromium(III) and chromium(VI) in a 50-ml calibrated flask, add 1 ml of 0.5 N sulphuric acid and 1 ml of 0.25% of diphenylcarbazide solution, mix and dilute to the mark with water. Measure the absorbance in a 1-cm cell at 540 nm using water as the reference. Calculate the chromium(VI) concentration, $C_{Cr(VI)}$, from a calibration graph constructed by taking aliquots containing known amounts of chromium(VI) in the range 0–50 μg per 50 ml of final solution through the procedure.

Destruction of the product of the reaction between chromium(VI) and diphenylcarbazide and of the excess of the reagent. Heat the resulting solution (above) in a boiling water-bath for 10 min and cool rapidly. The solution should be nearly colourless.

Determination of total chromium with 5-Br-PADAP. To 20-ml aliquots of the cooled colourless solution (above) add 2 drops of aqueous saturated 2,4-dinitrophenol solution, adjust the acidity of solution with 0.5 N sodium hydroxide until a red colour appears and neutralise with 0.5 N sulphuric acid until the red colour just disappears.

Add 7 ml of buffer solution and 5 ml of 0.04% 5-Br-PADAP solution and mix. Heat the solution in a boiling water-bath for 45 min and allow to cool. Add 1 ml of CTMAB solution, dilute to the mark with water and measure the absorbance [which corresponds to $A_{\text{Cr(III and VI) - 5-Br-PADAP}}$ per 2 ml of the test solution] in a 1-cm cell at 580 nm against a reagent blank.

Find the value of $A_{\text{Cr(VI) - 5-Br-PADAP}}$ from $C_{Cr(VI)}$ obtained in the first step and from the calibration graph constructed as follows.

Place amounts of standard solution to cover the range 0–50 μg of chromium(VI) in separate 50-ml calibrated flasks. Dilute to about 5 ml with water, add 1 ml of 0.5 N sulphuric acid and 1 ml of diphenylcarbazide solution, heat in a boiling water-bath for 10 min and allow to cool. Add 2 drops of 2,4-dinitrophenol, then 0.5 N sodium hydroxide solution dropwise until a red colour appears, then 0.5 N sulphuric acid until the red colour just disappears. Add 7 ml of buffer solution and 5 ml of 0.04% 5-Br-PADAP solution, heat the solution in a boiling water-bath for 45 min and allow to cool. Add 1 ml of CTMAB solution, dilute to the mark with water, then measure the absorbance in a 1-cm cell at 580 nm against a reagent blank.

Calculate $A_{\text{Cr(III) - 5-Br-PADAP}}$ from the following equation:

$$A_{\text{Cr(III and VI) - 5-Br-PADAP}} - A_{\text{Cr(VI) - 5-Br-PADAP}} = {}_{\text{5-Br-PADAP -}} A_{\text{Cr(III)}}$$

where A refers to the absorbance per 10 ml of test solution.

Finally, calculate the chromium(III) concentration, $C_{Cr(III)}$, from a calibration graph obtained by using known amounts of chromium(III) and developing the coloured complex with 5-Br-PADAP and measuring the absorbance at 580 nm, as above.

Results and Discussion

Study of Optimum Conditions for the Determination of Chromium(III) and/or Chromium(VI) with 5-Br-PADAP

Effect of heating time and temperature

Preliminary tests had shown that chromium(III) and/or chromium(VI) did not react with 5-Br-PADAP at room temperature, probably because of inertness of $[Cr(H_2O)_6]^{3+}$.

The absorbance of the complex varies with heating time and temperature, but a high and stable absorbance was obtained by heating the reactants in a boiling water-bath for 45 min and then cooling rapidly to room temperature (Fig. 1).

Effect of cetyltrimethylammonium bromide

If heating was carried out in the presence of CTMAB, a surfactant (*i.e.*, micellar solubilising agent), and then the solution was cooled rapidly to room temperature, the absorbance was higher and remained constant for at least 2 h (Fig. 2). Optimum improvement was obtained with 0.5–2.0 ml of 10^{-2} M CTMAB (Fig. 3). Therefore, 1.0 ml of the CTMAB solution was used in all subsequent determinations.

Effect of pH

Chromium(III or VI) forms a brownish red complex with 5-Br-PADAP in weakly acidic medium (pH 3–7) after heating the reactants in a boiling water-bath for 45 min. The volume of acetate buffer solution (pH 4) was not critical but its effect appeared to be most constant in the range 6–8 ml (Fig. 4). Therefore, 7.0 ml of buffer solution was used in all subsequent work.

Effect of reagent concentration

Fig. 5 shows that a volume of at least 5 ml of 0.02% 5-Br-PADAP was required for complete reaction; 7 ml of the solution were used subsequently.

Absorption spectra

The absorption spectra of the chromium - 5-Br-PADAP complex solutions were identical for both chromium(III) and chromium(VI), having an absorption maximum at 580 nm (Fig. 6) indicating the formation of a single complex. The absorbance of the reagent was insignificant at 580 nm, the bathochromic shift of the spectrum being about 130 nm on complex formation. All absorbance measurements were therefore made at 580 nm. The apparent molar absorptivity was calculated to be 2.64×10^4 l mol^{-1} cm^{-1} and the Sandell

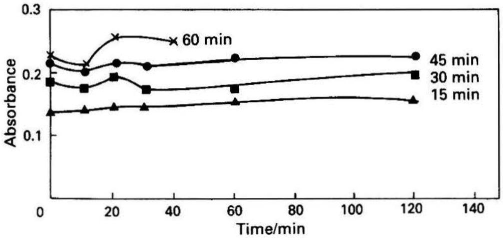

Fig. 1. Effect of heating time on the subsequent absorbance of the complex

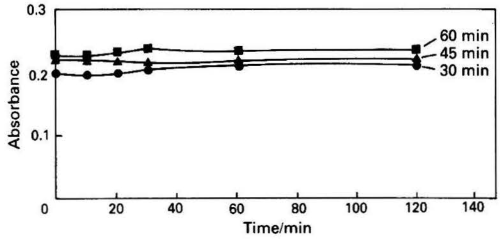

Fig. 2. Effect of heating time (60, 45 and 30 min) in the presence of CTAB on the stability and absorbance of the complex

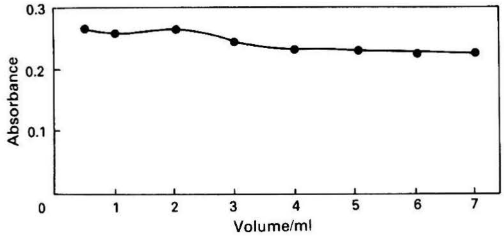

Fig. 3. Effect of concentration of CTAB on the absorbance of the complex

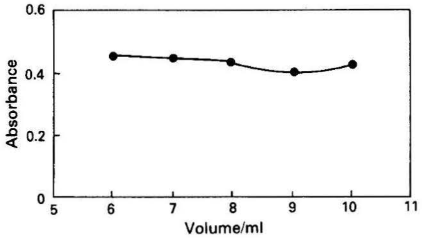

Fig. 4. Effect of pH on the absorbance of the complex

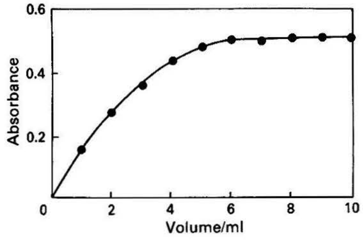

Fig. 5. Effect of amount of reagent on the absorbance of the complex

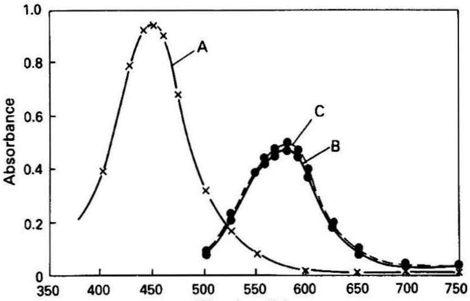

Fig. 6. Absorption spectra for A, reagent against water; B, chromium(III) - 5-Br-PADAP; and C, chromium(III) - 5-Br-PADAP complexes, both against reagent blank

sensitivity was 1.97 × 10^3 μg cm^{-2} of chromium for a net absorbance of 0.001.

Composition of the complex

The metal to ligand ratio in the complex was studied under the established working conditions by both Job's continuous variations method (Fig. 7) and the molar ratio method (Fig. 8). The molar ratio of chromium to 5-Br-PADAP was found to be 1 : 3 by both methods.

Validity of Beer's law

Under optimum conditions a linear relationship between absorbance and the amount of chromium(III) was found in the range 0–50 μg per 50 ml.

Interference study

The influence of foreign ions on the determination of chromium according to the procedure was investigated and the results are shown in Table 1. It can be seen that ions usually present in tap water and waste water do not affect the determination of chromium. Cobalt(II) interferes seriously and cannot be tolerated even at half the concentrations of chromium.

Re-examination of the Conditions for the Determination of Chromium(VI) with Diphenylcarbazide

Absorption spectra

The experiments showed that the absorption maximum of the product of the reaction of chromium(VI) with diphenylcarbazide occurs at 540 nm (ε = 3.12 × 10^4 l mol^{-1} cm^{-1}). This molar absorptivity is in agreement with some literature values[45] but lower than the normal figure of 4.10 × 10^4 l mol^{-1} cm^{-1}, probably owing to impurities in the diphenylcarbazide reagent (m.p. 162–165 °C).[46] Under constant experimental conditions, however, this does not have much effect on the accuracy and precision of chromium determination.

The reagent blank is negligible at 540 nm.

Adherence to Beer's law

Beer's law is obeyed from 2 to 50 μg of chromium(VI) per 50 ml of final solution. Chromium(III) does not affect the calibration graph for the determination of chromium(VI) with diphenylcarbazide.

Stability of diphenylcarbazide solution

The slope of the calibration graph obtained by using freshly prepared diphenylcarbazide solution was higher than that obtained when the solution had been stored.

Table 1. Effect of foreign ions on the determination of chromium with 5-Br-PADAP. Chromium(III) taken: 20 μg

Ion added	Tolerance ratio (ion : Cr)
Zr(IV), Nb(V), Ta(V), La(III)	1
Mo(VI), W(VI)	10
Mn(II), Zn(II), Hg(II), Bi(III)	12
Al(III), Pb(II), Cd(II), Sb(III), AsO_4^{3-}, PO_4^{3-}	50
K(I), Na(I), NH_4^+, Mg(II), Ca(II), Ba(II), Cl^-, NO_3^-, SO_4^{2-}	200
Sn(IV)*	1
Ti(IV)*	2.5
V(V)*	15
Ni(II)*	20
Fe(II or III)*	400
Cu(II)	1200

* Interferences eliminated by addition of 4 ml of 2 N hydrochloric acid to the final solution containing 20 μg of chromium(III) per 50 ml.

Destruction of chromium(III) - diphenylcarbazone complex and of excess of diphenylcarbazide

The elimination of the colour of diphenylcarbazide and the chromium(III) - diphenylcarbazone complex prior to the determination of total chromium with 5-Br-PADAP in an aliquot of the test solution could not be achieved by varying the acidity or by extraction with isoamyl alcohol. However, the colour can be simply destroyed by heating in a boiling water-bath for 10 min (Fig. 9).

Precision and Accuracy

The method has been applied successfully to the sequential determination of chromium(III) and chromium(VI) in tap water, chromium-plating electrolyte waste water and industrial waste water without prior separation. The relative

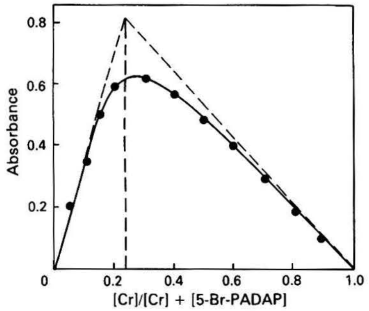

Fig. 7. Continuous variations graph for chromium - 5-Br-PADAP

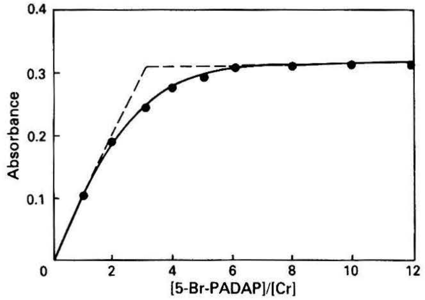

Fig. 8. Molar ratio graph for chromium - 5 Br-PADAP

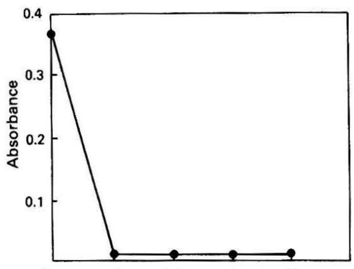

Fig. 9. Effect of heating time on the absorbance due to the chromium(III) - diphenylcarbazone complex in the presence of an excess of diphenylcarbazide

Table 2. Sequential spectrophotometric determination of chromium(III) and chromium(VI) in three water samples (n = 8)

	Added/µg		Found/µg		Mean recovery, %		Relative standard deviation, %	
Sample	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
Tap water A	0	0	2.1 ± 0.1	0			4.0	0
	10.6	20.0	12.8 ± 0.3	19.9 ± 0.4	101	99.5	1.33	1.10
Chromium-plating electrolyte waste water A*	0	0	3.7 ± 0.3	28.7 ± 0.5			4.05	1.03
	10.6	10.0	14.1 ± 0.6	39.0 ± 0.5	98.1	103	2.27	0.56
Industrial waste water	0	0	1.2 ± 0.2	0.5 ± 0			0.71	0
	10.6	20.0	11.3 ± 0.7	20.6 ± 0.6	95.3	101	3.19	1.65

* The pale green - yellow colour of the sample becomes almost colourless after diluting to the final volume with water.

Table 3. Comparison of the proposed method using 5-Br-PADAP with the diphenylcarbazide method

	Total chromium found/µg	
Sample	Proposed method	Diphenylcarbazide method
Tap water B	0	0
	0	0
	0	0
Chromium-plating electrolyte waste water B	1.6	1.5
	1.6	1.5
	1.6	1.5
	1.6	1.6

standard deviations, calculated from eight replicate analyses of each sample, were in the ranges 0.71–4.05% and 0.56–1.65%, respectively. The mean recoveries of added chromium(III) and chromium(VI) were 95.3–101% and 99.5–103%, respectively (Table 2).

The method was compared with a conventional diphenylcarbazide method; the results agreed satisfactorily (Table 3).

Conclusions

5-Br-PADAP is proposed as a photometric reagent for the determination of chromium(III) and/or chromium(VI). It forms a coloured complex with both chromium(III) and chromium(VI) without prior conversion of the valence state. Probably, 5-Br-PADAP is both a complexing agent for chromium(III) and a reducing agent for chromium(VI), so it can be applied conveniently to the determination of total chromium. The sequential determination of the total chromium with 5-Br-PADAP following the determination of chromium(IV) with diphenylcarbazide and destruction of the colour of the chromium(III) - diphenylcarbazone complex by boiling is feasible. The results of the determination for three samples showed that the precision and accuracy are satisfactory.

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Separation and Determination of Phenylhydrazine-*N*-dithiocarbamates of Ruthenium(III), Rhodium(III) and Palladium(II) from Other Group VIII Metals Using Thin-layer Chromatography and Visible Spectrophotometry

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Phenylhydrazine-*N*-dithiocarbamate forms coloured complexes with Ru(III) Rh(III), Pd(II), Fe(III), Ce(III), Ni(II), Ir(III) and Pt(IV). The dithiocarbamates of Ru, Rh, Ir and Pd are soluble in acetone whereas those of Fe, Co, Ni and Pt are insoluble. The separation of the phenylhydrazine-*N*-dithiocarbamates of Ru(III), Rh(III) and Pd(II) from other Group VIII metals and their determination has been carried out using TLC and visible spectrophotometry. Ru, Rh and Pd dithiocarbamates can be resolved successfully in chloroform, benzene, ethyl acetate - carbon tetrachloride (1 + 4) and acetonitrile - carbon tetrachloride (1 + 4). The coloured spots of the dithiocarbamates are visible in daylight and are the same in the different mobile phases.

Keywords: *Ruthenium(III), rhodium(II) and palladium(II) determination; phenylhydrazine-*N*-dithiocarbamates; visible spectrophotometry; thin-layer chromatography*

This work forms part of an investigation into the use of thin-layer chromatography (TLC) and spectrophotometry in the visible range for the quantitative separation and determination of ruthenium(III), rhodium(III) and palladium(II) as phenylhydrazine-*N*-dithiocarbamates in the presence of each other and iron(III), cobalt(III), nickel(II), iridium(III) and platinum(IV). The coloured spots of these metal dithiocarbamates are visible in ordinary daylight without any spray reagent.

Dithiocarbamates of transition and non-transition elements have previously been chosen as migrating species on thin layers of silica gel G.[1–7] Singh *et al.*[8] separated selenium(IV) and tellurium(IV) morpholine *N*-dithiocarbamates quantitatively from other metal dithiocarbamates using TLC and visible spectrophotometry. Some other workers have also used TLC to separate ruthenium, rhodium and palladium complexes with different organic reagents.[9–12] Different dithiocarbamates of ruthenium(III), rhodium(III), palladium(II), iridium(III) and platinum(IV) have been characterised.[13–19]

Owing to the lack of selectivity of precipitation of metal ions, these phenylhydrazine-*N*-dithiocarbamates interfere in gravimetric separations and determinations. Because of the rapid and better separability achieved, the combination of TLC with spectrophotometry has been utilised for the separation and determination of ruthenium, rhodium and palladium as phenylhydrazine-*N*-dithiocarbamates, which has not previously been attempted.

Experimental and Results

Apparatus and Reagents

Spectrophotometer

A Bausch and Lomb Electronic-20 spectrophotometer was employed for the absorbance measurements.

Solutions

All the solvents and reagents were of analytical-reagent grade.

Synthesis of the reagent

The potassium salt of the phenylhydrazine-*N*-dithiocarbamic acid was prepared by mixing potassium hydroxide, phenylhydrazine and carbon disulphide (1 + 1 + 1) in diethyl ether, while cooling in ice - water. The separated solid was filtered and washed with diethyl ether. A 1% *m*/*V* solution of the potassium salt of the reagent in distilled water was then prepared.

Solutions of metal ions

A solution of ruthenium(III) chloride, rhodium(III) chloride, palladium(II) chloride, iridium(III) chloride (hydrated) and chloroplatinic acid (from Johnson Matthey) was prepared by dissolving 1 g of the compound in 100 ml of distilled water in the presence of 2 ml of dilute hydrochloric acid. A solution of nickel(II), cobalt(II) and iron(III) was prepared by dissolving the required amount ($0.01\ \mathrm{mol\ l^{-1}}$) of their sulphates in 100 ml of distilled water in the presence of 1 ml of dilute acetic acid.

Buffer solution

An acetic acid - sodium acetate buffer of pH 4.8 was used.

*Isolation of phenylhydrazine-*N*-dithiocabamate complexes*

The complexes were isolated in an aqueous medium by mixing the solutions of the reactants in stoicheiometric ratios (ligand in slight excess). The complexes were digested at 60 °C for 15 min, washed with distilled water and then diethyl ether and dried at 110–120 °C. With cobalt(II) phenylhydrazine-*N*-dithiocarbamate, rapid oxidation of Co(II) to Co(III) took place. All the complexes, when wet, gave red colours with chloroform.[20]

Standard solutions of the complexes were prepared in acetone [Ru(III), Rh(III), Pd(II) and Ir(III)], chloroform [Co(III) and Fe(III)] or dimethylformamide [Ni(II) and Pt(II)].

Procedure

Preparation and development of the plate

Silica gel G (E. Merck) was used for preparing 0.25-mm layers on the plates. The plates (150 × 100 nm) were dried at 100 °C for about 2 h in an oven. The solutions of anhydrous complexes in acetone (Ru, Rh, Pd and Ir), chloroform (Fe and Co) or dimethylformamide (Ni and Pt) were spotted on the dry plates by using a marked fine capillary. The spotted plates were placed in a chamber saturated with solvent vapour and developed by the ascending technique until the solvent front had travelled 100 mm. The spots of the complexes were clearly visible and no spray reagent was required to make them visible.

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The dithiocarbamates were analysed for carbon, hydrogen and nitrogen and the results are given in Table 1.

Determination and Separation of Ru(III), Rh(III) and Pd(II)

A mixture of metal dithiocarbamates of Group VIII elements was extracted with a known volume of acetone. The dithiocarbamates of Ir(III), Ru(III), Rh(III) and P(II) are soluble whereas those of Fe(III), Co(III), Ni(II) and Pt(IV) remain in the residue. Known volumes of standard solutions of the Ru(III), Rh(III) and Pd(II) complexes in acetone were spotted on silica gel G plates with a marked fine capillary. The plates were developed in the chromatographic chamber and the R_F values were calculated. The resolved spots of the metal dithiocarbamates were scraped off and extracted with 5 ml of acetone. The absorbances of the eluted complexes were measured at their λ_{max}. The absorbances of the same volume of Ru(III), Rh(III) and Pd(II) dithiocarbamates spotted on the chromatographic plate were also determined in 5 ml of acetone. The concentration of Ru(III), Rh(III) and Pd(II) metal dithiocarbamates was deduced from calibration graphs of concentration of metal dithiocarbamates *versus* absorbance. The results are given in Table 2.

Table 1. Elemental analysis of the dithiocarbamates. Values in parentheses are the calculated percentages

Complex	C, %	H, %	N, %
$Fe(PHDTC)_3$	41.35	3.75	11.08
	(41.81)	(3.99)	(12.22)
$Co(PHDTC)_3$	39.12	3.15	12.95
	(39.51)	(3.31)	(13.1)
$Ni(PHDTC)_2$	38.98	3.20	13.28
	(39.5)	(3.31)	(13.1)
$Ru(PHDTC)_3$	38.45	3.18	12.69
	(38.2)	(3.25)	(12.9)
$Rh(PHDTC)_3$	38.30	3.16	12.8
	(38.2)	(3.25)	(12.9)
$Pd(PHDTC)_2$	35.05	3.35	12.4
	(35.5)	(3.48)	(12.2)
$Ir(PHDTC)_3$	33.10	2.70	11.40
	(33.90)	(2.80)	(11.30)
$Pt(PHDTC)_3Cl$	31.08	2.68	10.85
	(32.3)	(2.71)	(10.76)

Effect of Foreign Ions

The R_F values of the complexes of Fe(III), Co(II), Ni(II), Ru(III), Rh(III), Pd(II), Ir(III) and Pt(IV) (Table 3) show that Ru(III), Rh(III) and Pd(II) complexes do not interfere with each other in chloroform, benzene, acetonitrile - carbon tetrachloride (1 + 4) or ethyl acetate - carbon tetrachloride (1 + 4). The separation of these metal dithiocarbamates was best in benzene, in which almost all of the dithiocarbamates of Group VIII elements have distinguishable R_F values. However, Co(III) (R_F = 0.98) interferes with Rh(III) (R_F = 0.98) and Ir(III) (R_F = 0.94),whereas in acetonitrile - carbon tetrachloride (1 + 4) Ni(II) (R_F = 0.55) interferes with Ru(III) (R_F = 0.50). To avoid the interference of Fe, Co, Ni and Pt, the mixture of dithiocarbamates is extracted with acetone, in which these metal dithiocarbamates remain insoluble, whereas the filtrate containing Ru, Rh, Pd and Ir dithiocarbamates is applied to the TLC plate and resolved in benzene. The low R_F value of the platinum complex in benzene and acetonitrile - carbon tetrachloride (1 + 4) might be due to the low solubility of the complex.

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

Table 2. Spot colours, $\lambda_{max.}$ and R_F values of complexes. Room temperature, 29 °C

			R_F value			
Complex	Colour of spot	$\lambda_{max.}$/nm	Chloroform	Benzene	Acetonitrile - carbon tetrachloride (1 + 4)	Ethyl acetate - carbon tetrachloride (1 + 4)
$Fe(PHDTC)_3$	Grey	520	0.53	0.61	0.60	0.60
$Co(PHDTC)_3$	Dark brown	530	0.98	0.30	0.90	0.90
$Ni(PHDTC)_2$	Green	490	0.35	0.30	0.55	0.45
$Ru(PHDTC)_3$	Black	470	0.75	0.80	0.50	0.70
$Rh(PHDTC)_3$	Yellowish green	500	0.98	0.95	0.85	0.85
$Pd(PHDTC)_2$	Yellowish green	510	0.85	0.70	0.75	0.95
$Cr(PHDTC)_3$	Yellow	440	0.94	0.40	0.40	0.80
$Pt(PHDTC)_3Cl$	Yellow	470	0.40	0.25	0.05	0.40
Development time/min . .			40	40	60	70

Table 3. Results for the determination of Ru(III), Rh(III) and Pd(II) with potassium phenylhydrazine-*N*-dithiocarbamate

Complex	$\lambda_{max.}$/nm	Amount of complex spotted/µg	Absorbance of complex in 5 ml of acetone	Absorbance of eluted complex in 5 ml of acetone	Amount of complex found/µg	Error, %
$Ru(PHDTC)_3$	470	485	20×10^{-3}	21×10^{-3}	486	+0.20
		490	20×20^{-3}	21×10^{-3}	486	−0.81
		485	20×10^{-3}	22×10^{-3}	487	+0.42
		490	25×10^{-3}	22×10^{-3}	487	−0.61
$Rh(PHDTC)_3$	500	548	50×10^{-3}	47×10^{-3}	543	−0.91
		540	45×10^{-3}	43×10^{-3}	538	−0.37
		545	48×10^{-3}	50×10^{-3}	548	+0.73
		540	45×10^{-3}	42×10^{-3}	535	−0.75
$Pd(PHDTC)_2$	510	375	81×10^{-3}	75×10^{-3}	360	−1.30
		368	78×1^{-3}	74×10^{-3}	364	−1.08
		360	75×10^{-3}	78×10^{-3}	368	+0.22
		375	81×10^{-3}	78×10^{-3}	368	−1.90

Discussion

The R_F values of the metal complexes of phenylhydrazine-*N*-dithiocarbamates given in Table 3 show that Ru(III), Rh(III) and Pd(II) complexes can be resolved successfully in chloroform, benzene, ethyl acetate - carbon tetrachloride (1 + 4) and acetonitrile - carbon tetrachloride (1 + 4). A tailing effect was observed when butanol, methanol and acetonitrile were used. When a mixture of the metal dithiocarbamate complexes is applied to the plate, the R_F values are very close to those obtained for a single metal dithiocarbamate. Also, the colour of the spot seems to be the same in different mobile phases. The presence of a single spot of each metal dithiocarbamate on the TLC plate confirms the formation of a single complex in each instance.

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Determination of Olaquindox in Medicated Animal Feeds by High-performance Liquid Chromatography

Analytical Methods Committee*
Royal Society of Chemistry, Burlington House, Piccadilly, London W1V 0BN, UK

Keywords: *Olaquindox determination; medicinal additives; animal feeds; high-performance liquid chromatography*

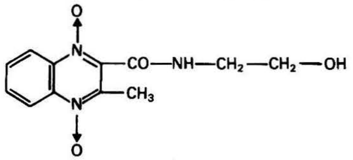
The Analytical Methods Committee has received and has approved for publication the following report from its Medicinal Additives in Animal Feeds Sub-Committee (B).

Report

The constitution of the Sub-Committee responsible for the preparation of this report was: Mr. P. Sanderson (Chairman), Mr. A. Anderson, Mrs. C. M. Billingshurst, Mr. C. G. Clifford, Mr. K. T. Chisnell, Mr. A. G. Croft (until June 1983), Dr. N. T. Crosby, Mr. G. Drewery, Dr. M. J. Gliddon, Mr. E. Goodall, Mr. R. S. Hatfull (until October 1982), Mr. G. H. J. Merson (until January 1983), Mr. I. Milner (from October 1982), Miss A. M. Moore (from October 1982), Dr. J. R. Salmon and Mr. M. R. Woolley with Mr. J. J. Wilson as Secretary.

Introduction

Olaquindox (**I**) {2-[*N*-2-(hydroxyethyl)-carbamoyl]-3-methylquinoxalin-1,4 dioxide} is a growth promoter that is added to animal feeds in amounts up to 100 mg kg^{-1}.



I

Experimental

In order to carry out a preliminary trial of the method, members were supplied with three samples of pig feed, a blank and two prepared by medicating this blank at approximately 25 and 50 mg kg^{-1}, respectively. They were also provided with details of a method, which was substantially that given in the Appendix. This method called for chromatography on a 10-cm column of 5-μm Partisil; some laboratories that did not have this column substituted the nearest equivalent that they had available. The results obtained are given in Table 1. Typical chromatograms are shown in Fig. 1. It was reported that olaquindox in solution was very unstable in both natural and artificial light and that care should be taken to protect these solutions from light.

These results were considered sufficiently promising to warrant a collaborative test using the method given in the Appendix. Five samples were supplied to each laboratory, these were: (i) a sample of a blank meal, (ii) this meal medicated with 25 mg kg^{-1} of olaquindox, (iii) material (ii) after pelleting and then regrinding, (iv) the blank meal medicated at 50 mg kg^{-1} and (v) material (iv) after pelleting and regrinding. Participants were asked to analyse each sample in duplicate on two separate days. They were also asked to spike the blank feed at 25 and 50 mg kg^{-1} and to analyse these samples in duplicate at the same time as they analysed the other samples.

The results obtained are given in Table 2.

Table 1. Determination of olaquindox in medicated pig feed pellets

	Olaquindox content/mg kg^{-1}						
Laboratory	Blank feed	Nominal level 25 mg kg^{-1}		Mean	Nominal level 50 mg kg^{-1}		Mean
1	1.5	29.7		30.0	56.7		57.3
		30.3			57.9		
2	—	26.0		26.0	54.0		54.0
3*	N.d.†	25.5		26.4	49.5		49.8
		27.3			50.0		
4	N.d.	31.8	30.0	30.5	55.7	54.9	55.8
	N.d.	30.4	29.8		55.9	56.7	
5‡	N.d.	25.7		25.7	50.6		50.6
6	0.8	31.5		31.5	58.6		58.6
7	—	30.7		30.7	57.4		57.4
8	—	31	31	31.0	61	61	
		31	31		60	60	60.5
Over-all number of laboratories				8			8
Mean/mg kg^{-1}				29.0			55.5
Standard deviation/mg kg^{-1}				2.5			2.5
Coefficient of variation, %				8.6			6.8

* This laboratory used a 25-cm Partisil 10 column and a flow-rate of 3.5 ml min^{-1}.
† N.d. = not detected.
‡ This laboratory used a 20-cm Zorbax Sil column: two columns, one commercially packed, the other packed in the laboratory gave similar chromatograms.

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The results for the spiked samples at each level of 25 and 50 mg kg^{-1} are all shown as percentage recoveries. The results on the medicated feeds are recorded in mg kg^{-1}.

One laboratory carried out a test for possible interference from other medicinal additives. A test feed sample was prepared by adding approximately 50 mg kg^{-1} each of acinitrazole, amprolium, arprinocid, buquinolate, clopidol, carbadox, decoquinate, diaveridine, dimetridazole, dinitolmide, ethopabate, furazolidone, halofuginone, ipronidazole, lasalocid, methyl benzoquate, narasin, nifursol, nitrovin, pyrimethamine, robenidine, ronidazole, sulphadimidine, sulphaquinoxaline, sulphanitran and furaltadone to a blank feed. Olaquindox (15.5 mg kg^{-1}) was then added to this feed and a portion of the mixture taken through the recommended procedure. It was found that nitrovin was the only additive that interfered with the determination of olaquindox.

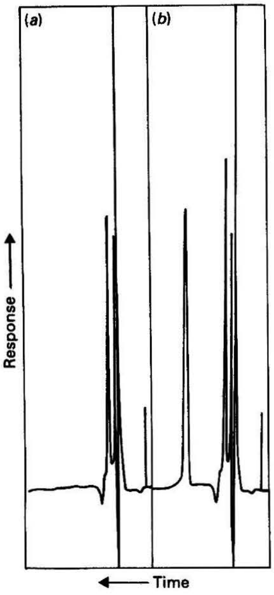

Fig. 1. Typical chromatograms of: (*a*) blank feed; and (*b*) feed spiked with olaquindox. Flow-rate, 2 ml min^{-1}; chart speed, 5 mm min^{-1}

Discussion

A statistical analysis of the results was carried out using Duncan's Multiple Range Test and this is summarised in Tables 3 and 4.

In Table 3 the mean recoveries for each laboratory are ranked in increasing order of magnitude. The means covered by a vertical line were not significantly different, which, at P = 0.05, would need to have been greater than 5.1. The over-all recovery was 94.9% with a coefficient of variation of 5.4%. There was no significant difference (P = 0.05) between the recoveries for the two levels of spiking.

In Table 4 the over-all mean recoveries for each laboratory covering the two levels of medication for both meal and pellets are ranked as described previously. The significant difference (P = 0.05) between two laboratories was 2.0 mg kg^{-1}, which is extremely low and produced significant differences between a number of laboratories.

The recovery for the meal samples was 103.5%, but for the pelleted material this was reduced to 92.0%, which was significant (P = 0.05).

The percentage recovery of the method was 97.9 ± 15.4 (P = 0.05) with a coefficient of variation of 7.7%.

Recommendation

The Analytical Methods Committee recommends the use of the method given in the Appendix for the determination of olaquindox in medicated animal feeds.

Table 2. Olaquindox content of medicated feeds

Laboratory	Recovery of spiked blank feed, %				Amount found in medicated feed containing 25 mg kg^{-1}/mg kg^{-1}				Amount found in medicated feed containing 50 mg kg^{-1}/mg kg^{-1}			
	25 mg kg^{-1} spike		50 mg kg^{-1} spike		Meal		Pellets		Meal		Pellets	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	100.0	95.6	98.0	90.4	35.1	32.3	33.0	30.0	68.1	62.3	54.3	52.0
	100.0	93.6	95.8	91.4	35.1	32.3	31.8	28.8	67.0	67.9	56.7	54.4
2	94.5	95.7	93.7	93.2	23.0	22.2	23.2	23.2	46.9	45.4	40.8	39.8
	95.7	93.0	95.5	93.2	22.2	20.4	23.2	22.2	48.5	46.8	42.3	41.2
3	94.0	97.6	95.5	96.1	23.8	25.7	22.3	23.2	45.8	48.3	39.2	39.7
	94.5	92.8	96.7	103.6	23.6	29.0	22.4	23.1	51.0	49.7	40.4	46.3
4	86.4	90.3	89.4	95.9	25.6	25.5	24.1	22.2	50.3	54.2	41.5	41.9
	88.0	90.3	89.4	90.7	25.3	24.1	22.7	23.5	53.0	49.6	45.3	42.8
5	87.8	116.9	94.2	109.8	23.7	27.4	23.6	26.5	56.8	58.4	45.0	50.9
	94.8	100.0	79.4	117.3	29.5	26.8	27.3	26.4	58.9	59.1	46.6	52.2
6	89.9	87.7	91.6	88.1	21.0	22.7	23.1	21.9	48.2	50.2	41.8	41.3
	91.4	85.1	92.3	88.7	23.8	21.8	24.1	20.1	48.5	44.4	44.0	40.3
7	97.0	97.0	95.0	99.0	23.0	24.0	21.0	22.0	43.0	48.0	41.0	40.0
	96.7	97.0	98.0	97.0	24.0	24.0	22.0	22.0	46.0	47.0	41.0	39.9
8	100.0	98.9	98.6	100.0	26.5	26.2	25.6	25.1	53.2	52.4	44.7	44.4
	98.9	99.3	99.3	99.3	27.1	25.8	24.9	25.1	51.4	49.9	45.3	45.2
9					21.4	23.5	25.0	25.1	53.6	51.4	46.4	44.7
					21.4	24.6	25.0	24.6	53.6	52.5	46.4	45.8
Mean	95.0		95.5		25.4		24.4		52.3		44.6	
Number of analyses	32		32		36		36		36		36	

Table 3. Recovery of olaquindox from spiked samples

Laboratory	Mean recovery, %	
6	89.3	
4	90.1	
9	91.9	
2	94.3	
1	95.6	Significant difference
3	96.3	$(P = 0.05) = 5.1$
7	97.0	
8	99.3	
5	100.0	
Number of results . .	72	
Over-all mean, % . .	94.9	
Standard deviation, %	5.1	
Coefficient of variation, % . .	5.4	
Recovery at 25 mg kg^{-1} level, %. .	94.6	Significant difference
Recovery at 50 mg kg^{-1} level, %. .	95.1	$(P = 0.05) = 2.4$

Table 4. Determination of olaquindox in circulated samples

Laboratory	Mean/mg kg^{-1}	
7	32.9	
2	33.2	
6	33.6	
3	34.6	Significant difference
4	35.7	$(P = 0.05) = 2.0$
9	36.6	
8	37.1	
5	39.9	
1	46.3	
Number of results . .	144	
Over-all mean/ mg kg^{-1} . .	36.7	
Standard deviation/ mg kg^{-1}	2.8	
Coefficient of variation, % . .	7.7	
Recovery, %	97.9	
Recovery from meal samples, %	103.5	
Recovery from pellet samples, %	92.0	Significant difference
Recovery at 25 mg kg^{-1} level, %. .	99.6	$(P = 0.5) = 0.9$
Recovery at 50 mg kg^{-1} level, %. .	96.8	

APPENDIX

Determination of Olaquindox in Medicated Animal Feeds

Purpose and Scope

The method is for the determination of olaquindox in animal feeds containing up to 100 mg kg^{-1} of additive.

Principle

Olaquindox is extracted from the feed sample by shaking with a mixture of acetone and water. The feed material is then filtered off on a glass-fibre filter and the olaquindox content of the filtrate determined by HPLC using a detector at 393 nm. The height of the olaquindox peak is measured and compared with that given by a solution of olaquindox of known concentration.

Note—Solutions of olaquindox decompose rapidly when exposed to daylight. Amber glassware or glassware covered in aluminium foil or black tape should be used where appropriate and all operations carried out in a dark room with safety light or in subdued daylight.

Reagents

Acetone.

Acetic acid (glacial).

Ethyl acetate.

Methanol.

Eluting solution. Mix 920 ml of ethyl acetate, 80 ml of methanol and 1.0 ml of acetic acid.

Extraction solvent. Dilute 800 ml of acetone to 1 l with water, mix well and allow to cool.

Olaquindox, analytical standard.

Olaquindox, standard solution. Weigh accurately about 10 mg of olaquindox into a 100-ml amber glass calibrated flask, add about 60 ml of solvent, shake to dissolve the olaquindox, make up to volume with solvent and mix well. Transfer by pipette 20.0 ml of this solution into a 100-ml amber glass calibrated flask, dilute to volume with solvent and mix well.

Apparatus

Liquid chromatograph. Fitted with a variable wavelength UV detector having a low (10 µl) dead volume flow cell.

Column. Stainless steel, 10 cm × 5 mm i.d., packed with Whatman Partisil (5 µm) or equivalent material.

Sample injection valve, 20 µl.

Glass-fibre filter. Whatman GF/F.

Amber glass calibrated flasks, 100 ml.

Typical chromatograph operating conditions

Typical conditions were as follows: temperature, ambient; flow-rate, 1.3 ml min^{-1}; detector wavelength, 393 nm; pressure, approximately 400 lb in^{-2} (or sufficient to achieve the required flow-rate); and attenuation, 0.1 absorbance unit full scale.

Procedure

Weigh (to 0.01 g) about 20 g of finely ground sample into a 150-ml conical flask and add 100.0 ml of extraction solvent. Stopper the flask and shake mechanically for 1 h. This must be carried out in the dark or else the flask must be covered with black paper. Filter the solution through a glass-fibre filter, discarding the first 20 ml and retaining the subsequent filtrate.

Chromatograph 20 µl volumes of both the standard and sample solutions and measure the heights of the peaks due to olaquindox. Under the conditions given here the retention time of olaquindox is about 4 min.

Calculation of Results

The results are calculated as follows:

Amount of olaquindox (mg kg^{-1}) =

$$\frac{\text{Mass of standard (mg)}}{\text{Mass of sample (g)}} \times \frac{A}{B} \times 200$$

where A = height of olaquindox peak in sample and B = height of olaquindox peak in standard.

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SHORT PAPERS

Preparation of Voltammetric Disc Electrodes Using Graphite-loaded Epoxy-coated Fabric as a Master Source of Electrodes

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An alternative to the multi-layer coating method of preparing voltammetric disc electrodes is described, which uses a graphite-loaded epoxy polyester net fabric as a master source of the electrodes. The smoothness of the coated fabric surface was improved by polishing with slurries of alumina. Linear sweep voltammograms are very similar to those given previously for this type of surface, which is smooth and homogeneous and gives robust discs.

Keywords: *Graphite-loaded epoxy-based electrodes; hydrofluoric acid vapour hardening; voltammetry; disc electrodes*

Previously, disc and coated wire electrodes[1] and coated plastic rod electrodes[2] were prepared using a new multi-layer coating and hardening technique. The surface was coated thinly and smoothly with a graphite-loaded epoxy base containing no hardener. The surface was then placed in a vapour chamber over 40% *m/m* hydrofluoric acid for 5 min before being removed from the chamber and the epoxy coating was hardened at 50–60 °C by the hydrofluoric acid it had absorbed. The process was repeated several times to build up the electrode. With coated plastic surfaces[2] the graphite content of the first few coatings was increased by rubbing graphite powder into the hardened coating in order to increase the conductivity of the coating.

The disc electrodes described previously[1] were constructed from thick-walled glass capillary tubing. A graphite-loaded epoxy filler containing a small amount of 40% *m/m* hydrofluoric acid was pressed into the end of the tube to occupy a length of about 0.5 cm. Whilst holding the end of the tube against a suitable surface to prevent the epoxy filler escaping, electrical contact was made to the filler by inserting a copper wire into it from the other end of the tube. The filler at the end of the tube was smoothed off by carefully rubbing the surface on a Cellophane sheet and the glass tube was cleaned with a soft tissue. The filler hardened in 1 h at room temperature and the surface was then polished with slurries of coarse and fine alumina, washed with water and dried. Three layers of graphite-loaded epoxy were then coated on to this surface and were hardened using hydrofluoric acid vapour. After the application of each layer the surface was polished on a filter-paper, washed with distilled water and dried. This produced a very smooth and shiny electrode surface. The electrodes were tested using linear sweep voltammetry and were shown to give highly satisfactory background currents and linear sweep voltammograms.

An alternative method of producing disc electrodes is described in this paper.

Experimental

Graphite-loaded Epoxy Base

This was prepared as described in the preceding paper.[3]

Graphite-loaded Epoxy Resin

This was prepared as described in the preceding paper.[3]

Isolating Epoxy Resin

This was prepared as described elsewhere (p. 27).[3]

Caution—Extreme precautions and care must be taken when using hydrofluoric acid. Work should be carried out in a fume-cupboard using the relevant safety equipment. Care must also be taken not to handle directly epoxy surfaces that may have retained hydrofluoric acid.

Use of Gaseous Hardener

This technique has been described previously.[1] Essentially, a thin layer of graphite-loaded epoxy base is placed on the surface to be coated. The article is then suspended in a plastic container containing about 2 ml of 40% *m/m* hydrofluoric acid solution for about 5 min to allow the epoxy base to absorb some hydrofluoric acid vapour. The article is then removed and heated at about 50 °C over a hot-plate for 5 min to harden the coating. This can then be repeated to build up the surface.

Coating of Polyester and Other Plastic Net

Graphite-loaded epoxy sheet was prepared by coating polyester net fabric, *i.e.*, dressmaking fabric. Spreading of the graphite-loaded epoxy base on the fabric was carried out using a flat plastic or metal spatula of the same width as the strip of fabric. Later in this work a metal spatula coated with hardened graphite-loaded epoxy was used exclusively for this purpose. A layer of base was placed on one side of the fabric. This was hardened and then further coatings were built up on this first side. Later, layers were built up on the reverse side of the fabric. The smoothness of the final coated fabric surface was improved by polishing with slurries of coarse alumina (70–150 μm) and with very fine alumina (0.015–0.3 μm). This coated fabric constitutes a master electrode source.

Preparation of Disc Electrodes from the Coated Fabric Master Electrode Source

Electrodes were cut conveniently from the master electrode source by means of an office paper punch. Larger discs were cut out with scissors. Copper wire was soldered on to the back of a copper or brass disc; the copper disc was usually slightly larger than the graphite-loaded epoxy fabric disc to facilitate fixing. The graphite disc was then fixed on to the copper disc by means of graphite-loaded epoxy resin. The assembly was sealed into a suitable glass tube as shown in Fig. 1 using the isolating epoxy resin.

Testing of the Electrodes

The electrodes were tested in a similar manner to that used in previous studies of the use of hydrofluoric acid hardened

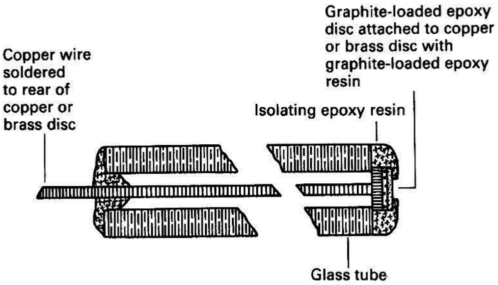
Copper wire soldered to rear of copper or brass disc
Graphite-loaded epoxy disc attached to copper or brass disc with graphite-loaded epoxy resin
Isolating epoxy resin
Glass tube

Fig. 1. Construction of disc electrode

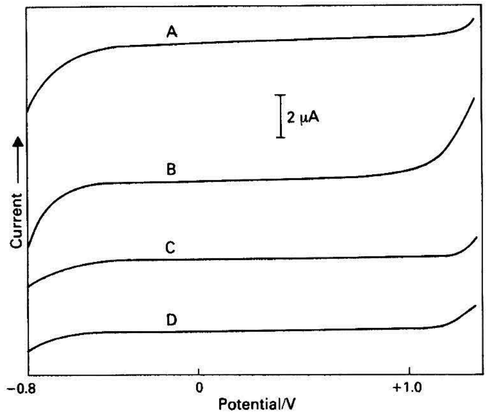

Fig. 2. Background currents obtained for linear sweep voltammetry. A and B, large disc; and C and D, small disc. Electrolyte: A and C, 0.18 M sulphuric acid; and B and D, pH 7.5 Britton - Robinson buffer solution. Sweep rate = 10 mV s^{-1}

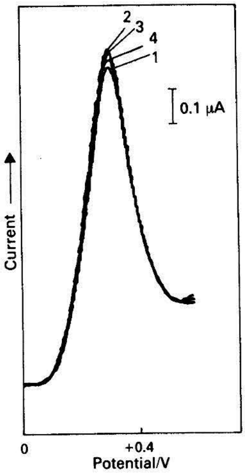

Fig. 3. Four consecutive differential-pulse voltammograms in dopamine. Scan speed = 5 mV s^{-1}; pulse amplitude = 50 mV. Cleaning in 1.5 M sodium sulphite solution at +1.5 V for 5 min between scans. The scan numbers are indicated

electrodes.[1–3] The background current was determined in the static mode in several electrolytes; slow linear sweep voltammograms (5–25 mV s^{-1}) and, in this instance, differential-pulse voltammograms were obtained for iodide, food colouring matters and dopamine. A three-electrode system was used with a Metrohm Polarecord 626.

Results

Numerous satisfactory electrodes of this type were prepared. Two sizes of electrode were constructed with active surfaces of 4 and 6 mm diameter prepared from epoxy discs 5 and 9 mm in diameter, respectively (see Fig. 1). Typical blank linear sweep voltammograms obtained in 0.18 M sulphuric acid and in pH 7.5 Britton - Robinson buffer solution are shown in Fig. 2. Clearly, very low background currents are obtained between about −0.4 and +1.0 V without deoxygenating the solutions.

The linear sweep voltammograms are very similar to those given previously for this type of surface.[1–3] Successive linear sweep voltammograms in 10^{-3} M potassium iodide in 10^{-1} M potassium chloride solution are identical. The electrodes responded as well as glassy carbon electrodes in food colour solutions when cleaned with ethanol, chloroform and carbon tetrachloride between scans. For example, a slight decrease in signal was observed for the new electrodes in tartrazine solution whereas there was a slightly greater decrease with a highly polished glassy carbon electrode. Adsorbed products from dopamine oxidations could be removed effectively by holding the electrode at +1.5 V in 1.5 M sodium sulphite solution for 2–5 min between scans. Differential-pulse voltammograms of dopamine for four successive scans at one of the smaller disc electrodes are shown in Fig. 3.

Discussion

An advantage of this type of disc electrode is that the disc itself is readily replaced. Numerous discs can be cut from the master source and these have virtually identical surface properties. The disc surface is very smooth and homogeneous, and can be polished very readily. The discs are very robust owing to the reinforcing action of the fabric net, and more physical pressure can be applied during chemical cleaning than with the disc electrodes described previously.[1] The previous disc electrodes can also be recovered by renewing the surface using the coating and hardening technique.

The discs are thin (*ca.* 0.2 mm) and the electrical resistance across them is small (*ca.* 30 ohm). The successive layers of graphite-loaded epoxy could be enriched with graphite powder to lower the resistance even more, as described previously,[3] but this does not appear to be necessary.

Other applications of the graphite-loaded epoxy-based fabric are being sought including its use in constructing voltammetric microcells.

H.P.H. thanks the University of Viçosa and CAPES (Brazil) for leave of absence and financial support.

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Amperometric Determination of Nitrite by Oxidation at a Glassy Carbon Electrode

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Nitrite ion has been determined amperometrically by oxidation at a glassy carbon electrode. A flow injection analysis procedure using this detector has been constructed and some interferences assessed. One of the interfering species was found to be ascorbic acid, and the analysis of an extract of a cooked meat was found to give satisfactory results when ion-interaction chromatography, with amperometric detection, was used.

Keywords: *Glassy carbon electrode; flow injection analysis; ion-interaction chromatography; nitrite determination; ascorbic acid*

Nitrite has been determined by flow injection methods using spectrophotometry[1,2] and by reduction procedures at a glassy carbon electrode.[3,4] The former methods depend upon colour formation during a diazotisation reaction and the latter depend upon reduction (at +0.30 V *versus* S.C.E.) in acidic bromide or chloride media. Work on the use of both glassy carbon and carbon paste electrodes for the oxidative determination of nitrite has already been published.[5] In this work both the flow injection analysis conditions and the electrochemical conditions for the determination of nitrite in a background of nitrate eluent have been optimised. Interference was observed from both chloride and ascorbate ions and a chromatographic method was employed to analyse samples such as meat extracts, where both of these ions are likely to be present.

Experimental

Apparatus

Eluent flow was controlled by a Gilson Minipuls-2 peristaltic pump and flow-rates of 0.86 cm^3 min^{-1} were monitored by a flow meter (Phase Separations, Clwyd). Injections were made via a Tecator valve fitted with a by-pass and a 30-µl sample loop. The detector cell was a Metrohm E641, which was set up on the wall-jet principle with a glassy carbon electrode and an Ag - AgCl reference electrode and fitted with a constant-temperature water-jacket (23 °C) and enclosed in an earthed metal box. Signals from the potentiostat were processed by a Spectra-Physics 4100 computing integrator. A Tacussel EPL-1 polarograph was also used for part of the initial investigation into the optimum potential. The chromatographic analysis was carried out with a Spectra-Physics 8700 solvent delivery system fitted with a 10-µl loop injector, and used a Hamilton PRP-1 poly(styrene - divinylbenzene) copolymeric column.

Solutions

The following set of solutions were prepared: (*i*) a stock solution of 1000 p.p.m. sodium nitrite in water; (*ii*) 100 cm^3 of (*i*) diluted to 1 dm^3 with water (100 p.p.m.); and (*iii*) 10 cm^3 of (*ii*) diluted to 1 dm^3 with 2 g dm^{-3} of potassium nitrate solution (1 p.p.m.). From the 1 p.p.m. sodium nitrite solution (*iii*), test samples were prepared by diluting 5, 10, 15, 20, 25, 30 or 40 cm^3 to a total of 50 cm^3 with 2 g dm^{-3} of potassium nitrate solution.

Meat Analysis

The PRP-1 column was conditioned for at least 1 h prior to use by passing (at a flow-rate of 1 cm^3 min^{-1}) a mobile phase of 17.5% acetonitrile and 82.5% aqueous (1 g dm^{-3}) *tert*-Bu_4NNO_3. Meat extracts were prepared according to the published standard procedure.[6]

Results

Flow Injection Analysis

Trials were carried out to establish both the optimum electrolyte concentration and the correct working potential of the glassy carbon electrode. Linear sweep voltammograms were recorded using a polarograph fitted with the electrodes from the wall-jet detector. No significant changes were observed in the voltammograms for electrolyte concentrations (potassium nitrate) in the range 2–20 g dm^{-3}, and so 2 g dm^{-3} was used for all subsequent measurements. A strong oxidation peak centred on 900 mV was observed (Fig. 1).

In the flow injection modes, a series of determinations of nitrite were performed for the test solutions of concentration 0.1–1.0 p.p.m. in sodium nitrite. For each solution about five separate responses were evaluated. The data were collected on a computing integrator and, although it would have been possible to process the information directly, this was not attempted. Instead, the peak heights and peak areas corresponding to each injection were recorded and the data subsequently analysed by standard statistical methods.

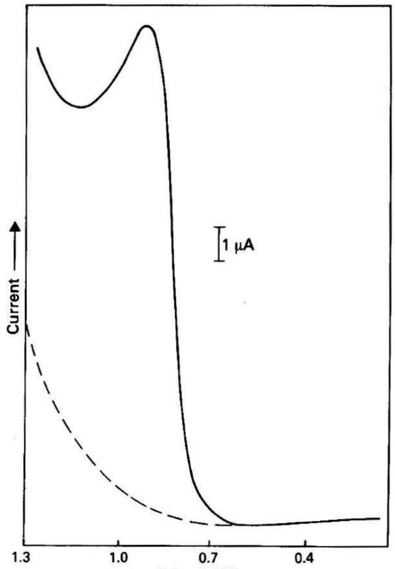

Fig. 1. Linear sweep voltammogram showing oxidation of nitrite ion at a glassy carbon electrode

Table 1. Statistical analysis of flow injection peaks obtained in the determination of nitrite standards. The data for peak heights and areas, in arbitrary units, were fitted by regression analysis to a straight line, $p = m[NO_2^-] + c$, with the nitrite concentration expressed in p.p.m. The sample size involved was eight different solutions, each analysed five separate times. The error limits above are the standard deviations from the mean value

	m (p.p.m.)$^{-1}$	c
Peak area	88.8 ± 0.4	−0.53 ± 0.25
Peak height	73.2 ± 0.4	0.67 ± 0.24

The response graphs from both peak height and area were strictly linear with slightly better results being obtained from the use of peak area values than the peak heights more commonly employed in flow injection analysis (Table 1). Thus there was a smaller intercept, and a smaller (relative) deviation in the slope when using peak areas.

Taking the chromatographic definition of detection limits[7] as being the amount of material required to produce a response equal to that of the detector noise, the detection limit was found to be 0.6 ng of sodium nitrite. As this was contained in 30 µl of solution, the minimum detection sensitivity is less than 0.02 p.p.m. of sodium nitrate.

The method as developed is suitable for the determination of nitrite in aqueous solutions containing a range of other species. Interferences were assessed by injecting 10^{-3} mol dm^{-3} samples of test ions dissolved in 2 g dm^{-3} of potassium nitrate solution. The results are as follows: no effect observed, Na^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Zn^{2+}, UO_2^{2+}, SO_4^{2-} and PO_4^{3-}; effects observed, NH_4^+, Mn^{2+}, Fe^{2+}, Co^{3+}, Pb^{2+}, Cl^-, I^-, PO_3^{2-}, $C_2O_4^{2-}$, SCN^-, SO_3^{2-} and ascorbate. No attempt was made to assess the relative sensitivity of the procedure to the different interfering ions. The electrochemical potential employed in this procedure is able to give some selectivity in detection, but there are clearly a fair number of species that will prevent the possible widespread application of this oxidative method.

The oxidation step does convey some advantages to the analyst, and amongst these is fairly high sensitivity and the elimination of the requirement to react with a halide species that is needed in the reported[3,4] reduction methods. The present detection limit of *ca.* 0.02 p.p.m. compares favourably with the reported value of *ca.* 10^{-7} mol dm^{-3} for that procedure. During the normal analysis there was no appreciable deterioration of the glassy carbon electrode over a comparatively long period of usage. However, for some of the interfering ions (*e.g.*, NCS^- and certain of the metal ions) it was necessary to polish the electrode after even one sample had been injected.

Chromatographic Analysis

Ion-interaction chromatography[8] was used to study some samples that contained nitrite ions and both chloride and ascorbate ions. This is a common situation as ascorbic acid is frequently added to preserved meat to assist in curing and colour retention.[9] It is also known that this substance will interfere with colour formation during the diazotisation methods for nitrite analysis.

Ion-interaction chromatography makes use of equilibria between the analyte anion and a co-anion in the presence of R_4N^+ counter ions on a non-polar copolymeric stationary phase (PRP-1). Selectivity between analyte anions is controlled by alteration of the co-anion in the eluting solution, changing the mobile phase composition and by the chain length of R in R_4N^+. The use of *tert*-Bu_4NNO_3 as the quaternary ammonium salt led to a reasonable retention time of 3.1 min for nitrite compared with less than 1.5 min for either chloride or ascorbate.

Using the perchlorate (R_4NClO_4) no separation of the three ions was observed (retention times *ca.* 1 min) and using R_4NSO_4 it took about 40 min to elute nitrite from the column. Hence the nitrate salt represents a good compromise between analytical speed and selectivity. A concentration of 2.7×10^{-3} mol dm^{-3} (in 82.5% water, 17.5% acetonitrile) of the salt also gave an acceptable noise level in the electrochemical detector.

In this mode of operation a calibration graph was constructed (using standard nitrite samples) and a detection limit of 2.2 ng (0.22 p.p.m. solution) established. This is approximately eight times less sensitive than found for the flow injection analysis method discussed above, but about three times higher than the 17 ng (in 20 µl) reported[10] for a similar procedure that used tetrapentylammonium bromide as the eluting salt and relied upon UV detection at 240 nm.

Comparative Test

A series of trials was undertaken to test the efficiency of the proposed procedures against that of a standard colorimetric method.[6] Some synthetic samples of nitrite - nitrate were prepared by fusion, having sodium nitrite contents from 10 to 50% *m/m*. This material was used to prepare standard solutions in the nitrite concentration range of 50–150 × 10^{-3} mol dm^{-3}. Reasonable agreement was attained between the flow injection method and the colorimetric procedure, with the latter showing a consistently higher value (+6%) than the former. Such agreement was not found when attempting the analysis of an extract from a cooked meat containing both nitrite and ascorbate ions. Analysis by the chromatographic procedure gave a level of 5.7 p.p.m. of sodium nitrite in the original ham, whereas the colorimetric procedure gave only 2.0 p.p.m. (on the same extract). This result shows clearly that the standard method can seriously underestimate the level of nitrite when found in conjunction with ascorbic acid. The chromatographic procedure effects a separation from other ions and allows a measurement to be obtained free from possible interferences.

M. P. Lopez de Haddad expresses her thanks to the Universidad de Oriente, Venezuela, for study leave.

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Simple and Sensitive Method for Monitoring Zopiclone in Plasma by High-performance Liquid Chromatography With Fluorescence Detection

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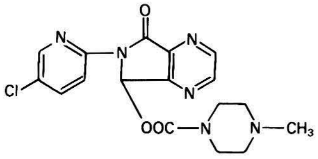
A reversed-phase HPLC - fluorescence method has been devised for the assay of the therapeutic plasma concentrations of the non-benzodiazepine hypnotic drug, zopiclone. A structural analogue of zopiclone is utilised as the internal standard. The method is reproducible, and using 2 ml of plasma has a lower detection limit for zopiclone of around 5 ng ml^{-1}. This procedure was used to follow the plasma level *versus* time curve of zopiclone for 10 h in a healthy volunteer given a single oral dose of 7.5 mg of zopiclone. A peak level of 86 ng ml^{-1} was achieved 1 h after dosing, and the apparent plasma elimination half-life of zopiclone was 4.9 h.

Keywords: *Zopiclone assay; high-performance liquid chromatography; fluorescence detection*

Zopiclone, {27267 R.P.; 6-(5-chloro-2-pyridyl)-7-[(4-methyl-1-piperazinyl)carbonyloxy]-6,7-dihydro[5*H*]pyrrolo[3,4,β]pyrazin-5-one; **I**} is a new non-benzodiazepine drug that has been shown in insomniac patients to possess hypnotic properties,[1] and has a rapid onset of action.[2] The optimum effective daily oral dose of zopiclone is 7.5 mg[3,4] and investigation of its pharmacokinetic properties[5,6] using normal-phase high-performance liquid chromatography (HPLC)[7], has indicated that plasma levels usually lie in the range 20–80 ng ml^{-1}. This paper describes a reversed-phase HPLC method for assaying zopiclone in plasma, which includes a structural analogue of zopiclone as an internal standard (29481 R.P.; **II**) and utilises their native fluorescence to give a lower detection limit for zopiclone of around 5 ng ml^{-1}.



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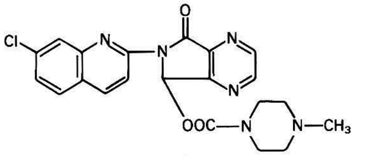

II

Experimental

Equipment

The HPLC system consisted of a reciprocating pump (Model 6000A, Waters Associates, Northwich, Cheshire, UK), a sample injector (Model 7125, Rheodyne, Berkeley, CA, USA) a fluorescence detector (Kratos Model FS 970, Schoeffel Instruments, Westwood, NJ, USA) set at a sensitivity of 0.1 μA and a dual-pen recorder (Model PM 8222, Philips, Eindhoven, The Netherlands). Quantitation was carried out by measuring peak-height ratios or peak area ratios using a computing integrator (Model SP 4200, Spectra Physics, St. Albans, Hertfordshire, UK).

Reagents

Acetonitrile and dichloromethane (HPLC grade) were obtained from Rathburn Chemicals (Walkerburn, Peeblesshire, UK). Isopropyl alcohol and diethyl ether (both of Pronalys grade) and anhydrous sodium sulphate and disodium hydrogen phosphate were supplied by May & Baker, Dagenham, Essex, UK. Zopiclone, the internal standard, and two potential metabolites of zopiclone, namely the *N*-1-oxide (29753 R.P.) and the *N*-1-desmethyl analogue (32273 R.P.), were obtained from Rhône-Poulenc, Paris, France.

Solvent Extraction Procedure

Aliquots of plasma (2 ml) were placed in glass centrifuge tubes and the internal standard (200 ng in 10 μl of acetonitrile) was added. The plasma was then extracted by shaking for 10 min with 10 ml of diethyl ether - dichloromethane (2 + 1) containing 1% *V*/*V* of isopropyl alcohol. Following centrifugation at 2500 rev min^{-1} for 5 min, the upper organic layers were transferred into tapered glass tubes. The extracts were dried with anhydrous sodium sulphate and then evaporated to dryness at 55 °C under a gentle stream of nitrogen (oxygen-free). Residues were reconstituted in 50 μl of acetonitrile before being chromatographed.

Chromatography

The extracts (20 μl) were injected on to a reversed-phase analytical column (15 cm × 4.6 mm i.d.) slurry-packed with Hypersil ODS (3 μm). The column at ambient temperature was eluted with 0.01 M disodium hydrogen phosphate (adjusted to pH 6.5 with orthophosphoric acid) - acetonitrile (150 + 80) that had been purged with helium. With a flow-rate of 1.5 ml min^{-1} the pressure was approximately 2500 lb in^{-2}. Zopiclone and the internal standard were detected by means of their native fluorescence (excitation wavelength 305 nm; emission filter 470 nm).[7] In order to extend the useful life of the analytical column, it may be protected with a small guard column (5 cm × 4.6 mm i.d.) containing Spherisorb S10 ODS (10 μm).

Specificity

In order to assess the specificity of the method, the chromatographic behaviour of two potential metabolites of zopiclone, namely the *N*-1-oxide and *N*-1-desmethyl analogues, were examined by the same procedure.

Results and Discussion

Using the proposed procedure, blank human plasma produced remarkably clean HPLC traces with no peaks in the

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* To whom correspondence should be addressed.

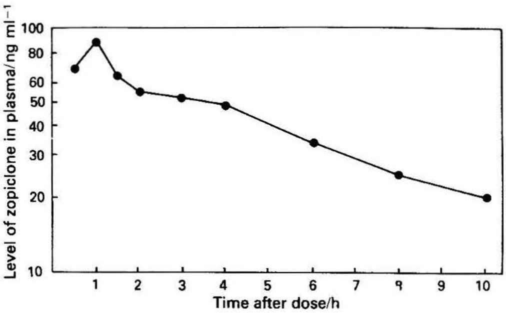

Fig. 1. Plasma level *versus* time profile for zopiclone in a healthy male volunteer given a single oral dose (7.5 mg) of zopiclone

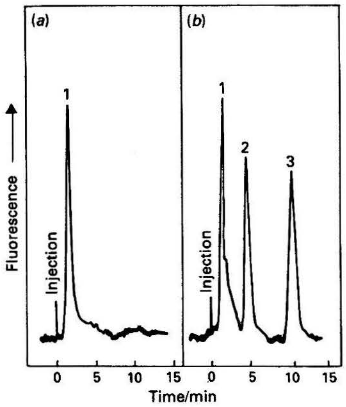

Fig. 2. Chromatograms of extracts of (*a*) blank human plasma and (*b*) human plasma containing zopiclone at 30 ng ml^{-1} and the internal standard at 100 ng ml^{-1}. Chromatographic conditions as given in text. Peaks: 1 = solvent front; 2 = zopiclone; and 3 = internal standard (29481 R.P.)

region of zopiclone [retention time (RT) 4 min] or of the internal standard (RT 9.5 min). Using 2 ml of plasma the detection limit for zopiclone is about 5 ng ml^{-1}. Calibration graphs were linear over the range from 0 to at least 200 ng of zopiclone per millilitre of plasma.

The reproducibility of the method was investigated by analysing ten replicate samples of plasma containing zopiclone at 10 or 30 ng ml^{-1}. The resulting within-batch coefficients of variation of peak-height ratios of zopiclone (to the internal standard) were 7.3 and 1.9%, respectively. The *N*-oxide analogue of zopiclone eluted very quickly and did not interfere with the zopiclone peak. However, some interference was produced by the *N*-desmethyl analogue of zopiclone, although a recent investigation[8] has shown that this potential metabolite of zopiclone is not present in the plasma of subjects given zopiclone. The possibility remains that some other, hitherto unidentified, metabolite of zopiclone might interfere with the assay.

The method described is rapid, appears to be valid for the parent drug in plasma and is sufficiently sensitive for monitoring therapeutic plasma levels. A typical plasma concentration - time graph obtained using this method for samples obtained from healthy volunteers given a single oral zopiclone dose of 7.5 mg is shown in Fig. 1. As can be seen, in this subject the peak plasma level of zopiclone of 86 ng ml^{-1} was achieved 1 h after dosing. Over the period 4–10 h after dosing, zopiclone appeared to have a plasma elimination half-life of 4.9 h. Hence the proposed procedure should prove useful in monitoring plasma levels of zopiclone achieved during clinical trials with the drug.

Typical chromatograms produced by the assay procedure are shown in Fig. 2.

The authors thank Dr. C. J. C. Roberts of the Clinical Pharmacology Unit, Department of Medicine, Bristol Royal Infirmary, for obtaining the plasma samples.

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Citric Acid as a Masking Agent for High Concentrations of Nickel in the Determination of Selenium by Hydride Generation Atomic-absorption Spectrometry

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Citric acid has a masking effect on nickel when present in the determination of selenium by the hydride generation - atomic-absorption technique. Satisfactory signals are obtained for 10 µg l^{-1} of selenium in the presence of 1600 mg l^{-1} of nickel(II). The determination of selenium in nickel samples is then possible, provided a suitable acid mixture is used for dissolution. However, the standard additions calibration procedure is still necessary.

Keywords: *Selenium determination; hydride generation - atomic-absorption spectrometry; nickel interference; citric acid masking effect*

Owing to the well known interference from nickel, hydride generation - atomic-absorption spectrometry has not been particularly useful for the determination of selenium in nickel samples. However, Fleming and Ide[1] noted that the interference from nickel was less pronounced when iron was present in the sample. They were able to determine selenium in low-alloy steel without interferences. Welz and Melcher[2] also found that selenium could be determined in steels in the presence of up to 2% of nickel. These observations have recently been examined in greater detail and it was found that when iron(III) and nitric acid were added to the hydrochloric acid sample solutions, nickel did not depress the selenium signals for up to about 100 mg l^{-1} of nickel.[3] Welz and Melcher explained this interesting observation by a reduction of iron(III) to iron(II) (and possibly also of nitrate) in preference to the reduction of nickel(II) to elemental nickel. However, at a nickel concentration of 1000 p.p.m. only 10% of the sensitivity, relative to that when nickel was absent, was obtained.[3] This is a low sensitivity when real nickel samples low in selenium are analysed, as the low content of selenium permits only a limited dilution of the dissolved sample. Nickel concentrations of 1000 mg l^{-1} or even higher must be expected in such instances.

Until now the only useful hydride generation method for the determination of selenium in nickel samples seems to be that of Welz and Melcher[4] in which nickel is removed by filtration as nickel hydroxide before volatilisation of the selenium.

In searching for an agent that could compensate, at least to a sufficient degree, for the interferences from very high concentrations of nickel, it was found that citric acid could be used for this purpose. The masking properties of citric acid on nickel(II) in the determination of selenium by the hydride generation - atomic-absorption technique have been examined in this work.

Experimental

Apparatus

A Perkin-Elmer Model 300 atomic-absorption spectrometer equipped with a discharge lamp for selenium and operated at 6 W was used. The selenium signals were measured at the resonance line of 196.0 nm and recorded on a Radiometer Servograph REC 51 recorder. The Perkin-Elmer MHS-10 hydride generation system was operated as recommended by the manufacturer.

Reagents

Nickel chloride solution, 2000 mg l^{-1}. Prepared by dissolving the calculated amount of $NiCl_2.6H_2O$ of analytical-reagent grade (Merck) in water. Concentrated hydrochloric acid (50 ml) of analytical-reagent grade (Merck) was added and the solution was diluted to 1 l.

Hydrochloric acid solution, 0.6 mol l^{-1}. A 50-ml volume of concentrated acid was diluted to 1 l.

Citric acid. Citric acid monohydrate, of analytical-reagent grade (Merck) was used.

Selenium(IV) standard solution, 5 mg l^{-1}. Prepared by diluting a 1000 mg l^{-1} solution made from sodium selenite of puriss. pro analysi grade (Fluka).

Sodium tetrahydroborate(III) solution, 3% *m*/*V*. Prepared by dissolving the salt of purum pro analysi grade (Fluka) in water containing 1% *m*/*V* of sodium hydroxide. The solution was filtered before use.

Procedure

Samples of 100 ml of all test solutions were prepared as follows. Appropriate volumes of nickel(II) solution were each added from a burette to 100-ml flasks. Selenium(IV) standard solution (200 µl) (5 mg l^{-1}) and the decided mass of citric acid were added. The flasks were filled to about 90 ml with hydrochloric acid (0.6 mol l^{-1}). After the citric acid had dissolved and the solutions had reached ambient temperature, the flasks were filled to volume with the hydrochloric acid and mixed.

Aliquots (10 ml) of each solution and three aliquots of each concentration were taken for analysis. The results presented in Figs. 1–3 are the means of three determinations.

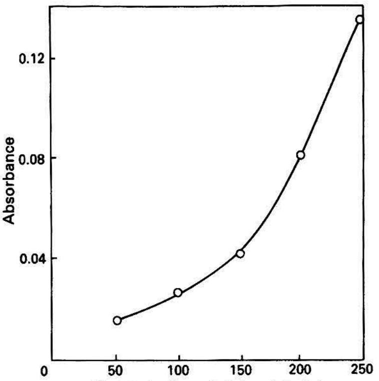

Fig. 1. Variation of peak height with concentration of citric acid in solutions containing 10 µg l^{-1} of Se(IV) and 1000 mg l^{-1} of Ni(II)

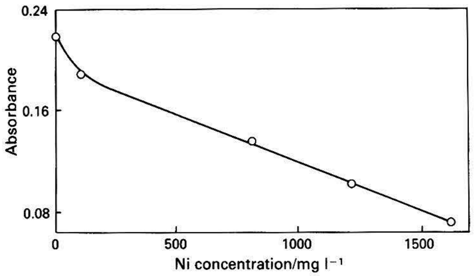

Fig. 2. Variation of peak height with concentration of Ni(II) in solutions containing 10 μg l^{-1} of Se(IV) and 250 g l^{-1} of citric acid

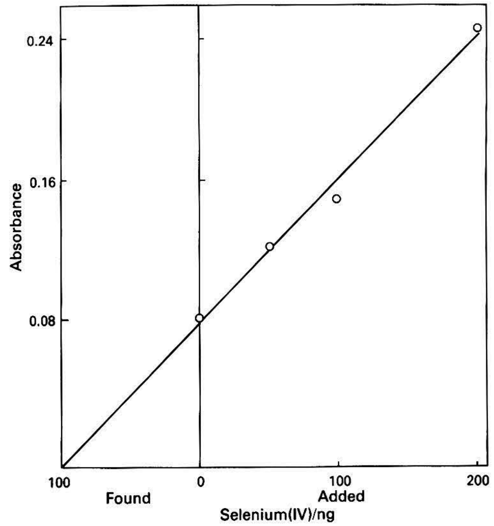

Fig. 3. Standard additions graph for 10 ml of solution containing 10 μg l^{-1} of Se(IV), 1600 mg l^{-1} of Ni(II) and 250 g l^{-1} of citric acid obtained 5 d after mixing

Results and Discussion

When 10-ml aliquots, containing different concentrations of citric acid, 10 μg l^{-1} of selenium(IV) and 1000 mg l^{-1} of nickel(II) were examined, it was observed that the peak heights increased with increasing concentration of citric acid, as shown in Fig. 1. Citric acid was not added at a concentration of above 250 g l^{-1} because of its limited solubility.

Considering that the first pK_a value for citric acid is 2.94,[5] and that the actual pH in the test solutions was about 0.2, it is questionable whether the masking effect of citric acid on nickel(II) can be attributed to a simple anionic complex formation only; in the literature it has been confirmed that citric acid does not form nickel complexes at pH values below 2.[6] A complete explanation of the masking properties of citric acid on nickel(II) in strongly acidic solutions is therefore uncertain.

In Fig. 2 it is shown that, although the highest possible concentration of citric acid is present, nickel(II) always depresses the selenium signals and that the degree of depression increases with increasing concentration of nickel(II). However, even at 1600 mg l^{-1} of nickel(II) the recorded absorbance for selenium is still very satisfactory for quantitative work (more than 30% of the signal obtained in the absence of nickel). Moreover, precipitation of nickel, which probably is the major contribution to the depression of the selenium signals, was never observed.

As a recovery test a solution containing 10 μg l^{-1} of selenium(IV), 1600 mg l^{-1} of nickel(II) and 250 g l^{-1} of citric acid was analysed some days after mixing using the standard additions procedure. The calibration graph obtained, illustrated in Fig. 3, shows the suitability of this procedure.

In utilising citric acid as a masking agent for nickel ions from dissolved nickel samples, attention should be paid to the presence of nitric acid used in digestion mixtures. Preliminary experiments indicated that nitric acid depresses the masking effect of citric acid. Attempts to find a suitable digestion procedure are now being undertaken in this laboratory.

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BOOK REVIEWS

Chemistry of Sulphur Dioxide in Foods

B. L. Wedzicha. Pp. xii + 381. Elsevier. 1984. Price £38. ISBN 0 85334 267 9.

This book is written by a specialist with over ten years experience of research in this field. The book is almost encyclopaedic in content and consists of seven chapters covering the chemistry, analysis, non-enzymic and enzymic reactions in food, enzymology, microbiology, food uses and toxicology of sulphur dioxide. Whilst the analytical aspects of sulphur dioxide will be of most interest to readers of this journal, the author is to be complimented on bringing together all the other important areas in such a critical and concise format. The book is undoubtedly the definitive and reference work in its field for both analytical chemists and food scientists.

The analytical chapter is a comprehensive review of qualitiative and quantitative methods for the identification and determination of S(IV) oxoanions. The advantages and limitations of both classical techniques and the more modern methods, such as molecular emission and ion-selective electrodes, are critically reviewed. The separation of S(IV) oxospecies by means of chromatographic techniques is also discussed together with the problems arising from the application of these methods to foodstuffs and model systems. The survey of methods available for the determination of reaction products in foods is particularly valuable.

Despite the fact that sulphur dioxide has been in use for many years as an important food preservative, our knowledge of the fundamental chemistry and reactions occurring in foodstuffs is still far from complete. This book is a timely review of the current progress of research in this important area of food science. The book is well produced and fully referenced with citations at the end of the book in alphabetical order.

N. T. Crosby

Regulatory Compliance Monitoring by Atomic Absorption Spectroscopy

Sidney A. Katz and Stephen W. Jenniss. Pp. viii + 278. Verlag Chemie International. 1984. Price DM 115. ISBN 0 89573 114 2 (Verlag Chemie International); 3 527 20658 7 (Verlag Chemie).

This book follows in the popular trend of publishing collections of "official" monitoring methods. That such compilations should be published is understandable, as a complete set of all seven volumes of the NIOSH Manual of Analytical methods, about 600 methods in all, will come out at around £1 per method.

In fact, the book is rather narrower than the title suggests, as the subject is mainly about North American methods for monitoring for compliance with North American environmental and workplace standards for metals in air and in water, soil sediments, wastes and biological samples. Further, it is mostly about analysis—by atomic-absorption spectroscopy.

The methods are given in condensed form in four of the eight chapters: Chapter 4 covers 32 of the NIOSH PCAM series for workplace exposure to 25 metallic elements; Chapter 5 is made up of 59 methods from the US EPA and the Canadian DOE for the analysis of 36 metals in water; Chapter 6 gives 29 methods from the same sources for 22 metals in wastes, sludges, sediments and soils, and gives a reasonable amount of detail on sample extraction; Chapter 7 has seven methods from either the NIOSH PCAM series or the Canadian DOE for the determination of 6 metals in biological samples such as urine, blood, fish and animal tissue. The remaining four chapters are aimed at putting the subject into perspective and giving background detail. The introduction is a short, superficial account of AAS followed by a short, but broad, coverage of sampling and sample preparation. The final chapter attempts to cover quality assurance, but is rather simplistic and has more "motherhood statements" than hard information.

It is difficult to sum up this book; it is unlikely to be of great interest to the environmentalist as it is too specific to the analysis of metals by AAS and so will mainly be for those AAS operators carrying out the analysis for the environmentalist, in which event the three chapters on the AAS technique, sampling and quality assurance are unlikely to be useful. Perhaps those in smaller industries who have to cover all the aspects will find the most use for this book.

As a compilation of AAS analytical methods for metals in environmental samples the book has achieved its main, but narrow, aim well enough but, as with much of North American documentation, no methods from the rest of the world receive a mention—even though, strangely, the West German Federal Health Department and the UK DOE are acknowledged for supplying procedures that I have been unable to find in this text. The "not invented here" syndrome lives on.

David T. Coker

Handbook of U.S. Colorants for Foods, Drugs, and Cosmetics. Second Edition

Daniel M. Marmion. Pp. xiv + 466. Wiley-Interscience. 1984. Price £47.50. ISBN 0 471 09312 2.

This is the Second Edition of this handbook and, like the First Edition, it is aimed mainly towards users in the USA. As previously, the book is divided into three parts dealing with: the history, regulation, description and uses of colouring material; the analysis of colouring matters themselves; and the resolution of mixtures and analysis of commercial products containing colouring matters.

The first part of the book provides much useful information on the colouring matters of interest to the user in, or exporter to, the US. Included are lists of certified colouring matters, their uses and Regulations governing such usage in the US. The structures and specification given for the individual colouring matters will be particularly valuable. The second part of the book deals in considerable detail with the identification and specific purity criteria for the colouring matters themselves. Although aimed towards the US reader, the UK user will also find this section of use. Many methods are given in this section and in sufficient detail for them to be used directly.

The third part of the book deals with both the resolution of mixtures of colours and the analysis of commercial products (*i.e.*, compound foods, cosmetics, etc.) for the colours incorporated into them. The methods are given in outline only in this part of the book together with the appropriate original references.

This book is very much an up-date of its First Edition. Those readers who found that First Edition of use will do likewise with this Edition. Analysts required to work in the area of colouring matters will find it of use, even though all the information given relates primarily to the US reader.

Roger Wood

Developments in Food Analysis Techniques – 3

Edited by R. D. King. *Developments Series.* Pp. x + 217. Elsevier Applied Science. 1984. Price £26. ISBN 0 85334 262 8.

This book is the third in a series devoted to a discussion of recent developments in food analysis procedures. This series is proving to be a useful addition to the bookshelves of food analysts as it forms a readily accessible reference work giving an initial understanding to various analytical techniques and of areas of analytical interest to such analysts. The five chapters included in this book are all written in review format; the book should therefore not be considered as a basic working aid to the analyst, although some sections do provide detailed descriptions of analytical procedures, but as a good source of referenced information.

One of the contributions to the book sets out to discuss a particular technique, that of immunochemical methods in food analysis, whereas the other four contributions discuss specific areas of interest to the food analyst, namely those of dietary fibre, trace elements, mycotoxins and pesticides.

Chapter 1 deals with the problems associated with the analysis of dietary fibre and gives a very good overview of this particularly complex area for the food analyst. It brings together and comments on many of the approaches that are used for this analysis, which is of particular interest at the present time. Chapter 2 is concerned with trace element analysis and gives a very brief overview of instrumental techniques. More interestingly, stress is laid on analytical quality assurance and data handling and evaluation, areas that are becoming of increasing importance to the food analyst.

Chapter 3 is concerned with the determination of mycotoxins and gives a good review of the present state of the art in this area. It also emphasises the increasing importance that immunoassay procedures have for these analyses. Chapter 4 deals with the analysis of pesticides in foodstuffs and is the most detailed, analytically, of the chapters in the book; there are only a limited number of references cited in this chapter. It gives the approaches recommended by the author of the chapter and which are not, therefore, exhaustive.

Chapter 5 is concerned with the use of immunochemical methods in food analysis and gives a good overview of such uses to those not familiar with them. The chapter serves as a useful introduction to this area of food analysis, which is of increasing importance, and gives a good list of references to specific problems to which immunochemical procedures have been applied.

This is a very readable and enjoyable volume in this series of books. Most of the contributions have provided a full list of references and so if the volume is used as a starting point these will guide the reader to more detailed information.

R. Wood

Optical Remote Sensing of Air Pollution. Lectures of a Course Held at the Joint Research Centre, Ispra (Italy), 12–15 April 1983

Edited by P. Camagni and S. Sandroni. Pp. x + 422. Elsevier. 1984. Price $100; Dfl260. ISBN 0 444 42343 5.

Titles can be deceptive; "Optical Remote Sensing of Air Pollution" suggests a comprehensive, structured treatment of the whole subject, a definitive work even. Sadly the Elsevier publication does not fulfil these expectations: the volume is very much oriented to the instrumentalist.

After a clear and competent explanation of the physical basis for optical remote sensing (Quenzel), the reader is treated to nearly 200 pages of erudite, some might say tedious, detail on instrument theory and construction. Millán, for example, has produced 42 such pages on correlation spectrometers before arriving at any practical advice. The following four pages then confirm the experience of many early enthusiasts: correlation spectrometry has rather limited field applications. Admittedly, from the same author, has come one of the better chapters in the second part of the book which attempts to cover practical applications and field experience of optical remote sensing in general. A further contribution on correlation spectrometry in this latter section (Sandroni) and two chapters on lidar (Capitini *et al.* and Anfossi) represent the most interesting parts of the volume. Although others nominally deal with the practical application of the remaining techniques, they are dominated by instrumental detail. A reader hoping for a review of operational experience would be rather disappointed.

The book as a whole lacks any discussion of the merits or otherwise of optical remote sensing over conventional monitoring systems or of the relative advantages/disadvantages of individual remote sensing techniques. One could be left with the feeling that perhaps the whole concept is more a development challenge for the instrument engineer rather than a practical tool for the routine study of air pollutants; but that would be cynical.

The quality of the volume is high, spoilt only by an inexcusibly high number of proof-reading errors, of which a publishing house like Elsevier should be ashamed. The chapter arrangement is, however, somewhat unstructured with no continuity in coverage of individual techniques. Probably the most extreme example is the contribution by Pettifer on the applications of optical remote sensing to meteorology. While this is a most interesting piece, its contents are somewhat unrelated to the study of air pollution and its location in the middle of the first (theoretical) half of the volume appears without logic. The publishers should also note that it is customary to provide authors' affiliations on the title page of each chapter rather than as a list after the Forward. At a price of £77 per copy, "Optical Remote Sensing of Air Pollution" is likely to have the market appeal of a Hebrew version of the New Testament.

R. A. Barnes

Methods in Enzymology. Volume 96. Biomembranes. Part J. Membrane Biogenesis: Assembly and Targeting (General Methods, Eukaryotes)

Edited by Sidney Fleischer and Becca Fleischer. Pp. lvi + 901. Academic Press. 1984. Price $79. ISBN 0 12 181996 5.

This volume is one of a series on methodology used to study membrane biogenesis, assembly, targeting and recycling. After a general and very readable introduction to membrane biogenesis the book is divided into two sections, the first considers the biogenesis and assembly of membrane proteins and the second selected techniques for studying the transfer of newly synthesised proteins, *i.e.*, targeting. In a sense, the coverage of the book is broad—from plant vacuoles to the biosynthesis of acetylcholine receptors—yet it consists of 65 papers describing detailed experimental and research work. As such it is therefore not possible to review it in great detail. The first 19 papers describe methods that might be of general use to those working in the membrane field. Detailed experimental instructions are usually given with appropriate guidance for those new to the techniques. The remaining papers are similarly detailed and well referenced but cover more specific aspects of membrane biogenesis.

This book is likely to be used or "dipped into" by workers in the field who will welcome the wealth of detail. It should be of value to the scientific reference library.

P. M. Clark

Handbook of Particle Sampling and Analysis Methods

Charles H. Murphy. Pp. xiv + 354. Verlag Chemie. 1984. Price DM165; $49.50. ISBN 3 527 26149 4.

This book is essential reading for anyone interested in the study of airborne particulates. Although intended for individuals with limited experience its coverage is so comprehensive that it will be of value also to the expert. The book begins with a brief, but informative, introduction, followed by a discussion of particle characteristics, units, dimensions and terminology.

This is followed by a section on particle sampling techniques covering inertial separation, filtration, condensation nuclei counting, diffusion, electrical mobility, light attenuation and scattering and miscellaneous techniques. The section closes with a chapter on sampling considerations and instrument calibration. This section enables the reader to select the most suitable method for any specific purpose. It comprises tables to summarise the methods, chapters in which the methods are described in detail and specifications of some commercially available instruments. Each chapter commences with a statement of the theory underlying the technique, together with a discussion on the limitations of the theory. This is followed by a discussion on the application of the theory to instrument design.

In the final part of the book methods of particle characterisation are presented. The two chapters on particle size analysis are well written but suffer due to their brevity. For example, on p. 256, it is stated that if a number distribution is log-normal the surface and volume distributions will also be log-normal, provided the particles are spherical. The actual requirement is that particle shape must be independent of size; also the distributions will not only be log-normal they will also have the same slope, *i.e.*, the same geometric standard deviation. These chapters are interesting as a concise statement of the subject but too brief to be comprehendable to anyone with a limited mathematical understanding or of value to anyone with the necessary background. Under these circumstances an expanded reference section would be useful. Optical microscopy (Chapter 15) is a useful technique for examining particles but, for particle sizing and counting, this technique has been largely superseded by automatic microscopy, which should have received a mention.

This section also includes chapters on the investigation of chemical composition using atomic-absorption spectroscopy, X-ray fluorescence, activation analysis and chromatography and a chapter on electron microscopy. Although, of necessity, brief, these chapters are well written and informative.

In summing up, Dr. Murphy deserves congratulation for an excellent book and any criticisms are minor compared with the plaudits with which it should be greeted.

T. Allen

Air Monitoring Methods for Industrial Contaminants

Edited by David A. Halliday. Pp. xii + 428. Biomedical Publications (distributed by John Wiley). 1984. Price £31.25. ISBN 0 931890 12 8.

This book is being issued at the same time that NIOSH are issuing their Third Editon of the "Manual of Analytical Methods." The book is a straight compilation of NIOSH methods for monitoring workplace exposure to around 200 substances, although it contains only about 80 complete methods, the others being adaptations of these, giving only the necessary changes in analytical details.

The new NIOSH Manual also contains methods for over 200 substances (102 complete methods) and so has a similar content to this book. In addition, the NIOSH Manual has sections on quality control, sampling, method development and analysis of biological samples and it is also in loose-leaf form and can be updated as new methods come along. As this book cost £31 and the NIOSH Manual $39 (in the USA), it should be obvious which is the better value.

In order to justify what would otherwise be a straightforward repackaging of methods, the Editor has tried to direct the reader towards the replacement of these methods by using portable (and sometimes fixed) direct-reading systems. Each method contains a table listing the measurement principle, capital cost, cost per measurement, concentration range and sampling duration for what is claimed to be "a compilation of the known commercial devices for quantitatively determining . . ." each substance. No supporting information, explanation or discussion is offered on this concept apart from a paragraph in the Preface.

This feature of the book fails miserably for several reasons. Firstly, the equipment listed is a very long way from being comprehensive, as in about 90% of the cases the devices listed for gases and vapours are only either detector tubes, the Miran portable IR, the HNU photoionisation detector or the Microsensor GC. Secondly, all the instruments mentioned have specificity, interference and response factor problems and a considerable amount of information is needed before they can be confidently used under field conditions—none is given. Thirdly, and most important, no mention is made of the fact that the NIOSH methods are for measuring personal exposure in the workplace, usually averaged over a whole workshift, but almost none of these instruments is suitable for that purpose. The only situations where direct-reading instruments are suitable for personal monitoring are the personal warning devices used for protection against insidious substances that can be immediately hazardous to health. There are only a handful of these in the book and their role is not discussed.

It is this aspect of the book that leaves one wondering if the Editor (about whom no information is given) understands the philosophy of workplace exposure monitoring. In the Preface he presents it more as a "non-essential luxury," impeding industrial profitability, than a legal and moral responsibility to protect the health of employees. The Editor hopes, in the Preface, that succeeding volumes will add those suppliers (a considerable number) inadvertently omitted from this volume. I would suggest that he should either publish a volume dedicated to direct-reading instruments or drop it completely.

David T. Coker

Steric Exclusion Liquid Chromatography of Polymers.

Edited by Josef Janča. *Chromatographic Science Series, Volume 25.* Pp. xvi + 329. Marcel Dekker. 1984. Price SwFr153. ISBN 0 8247 7065 X.

This Monograph, edited by Dr. Janča, brings together contributions from worldwide laboratories in the forefront of polymer steric exclusion analysis. It is eminently readable, clearly presented and contains a wealth of information useful to the practising chromatographer and indispensible to the researcher in the field of steric exclusion chromatography of polymers.

The complex and often only partly understood mechanisms of separation by steric exclusion chromatography (SEC) are dealt with in a lucid manner by Dr. Janča, who concedes that the method is "in view of the attained degree of knowledge, merely a separation method, itself rendering no absolute information on molecular weights, and their distribution, or on the structure of the polymers studied". All other information to be gleaned by the technique has to be performed by calibration.

Calibration, in all its diversities, is covered in the chapter by Dr. J. V. Dawkins, who describes and critically assesses the use of both narrow and broad relative molecular mass distribution samples, the use of and deficiencies in the so-called universal calibration method, and methods for calibration when no standard polymers of accurately known relative molecular mass are available.

A. E. Hamielec comprehensively reviews the vast field of axial dispersion, considering practical details that may contribute to spreading functions, mathematical solutions to the integral equation of the spreading function and numerical methods for the solution of the integral equation. Skewing of peaks in SEC is also considered in some detail.

The chapter on the effect of experimental conditions by Sadao Mori covers the effects of different column packings, concentration, flow-rate, interactions with solvents, the effects of incompatibility between solute and gel, injected volume, temperature and arrangement of columns.

The fascinating chapter by Claude Quivoron on the use of SEC for polymer analysis, deals with general mathematical procedures for data treatment both with single and coupled detectors. Means of measuring chain branching, and for determining chemical composition are reviewed, as are some of the more unusual applications of SEC, such as its use for studying chain growth mechanisms during polymerisation, chain degradation, particle size analysis, pore size analysis, polymer additive analysis and dimensional or interactional properties of polymers in solution.

A relatively brief but important chapter by Stenlund and Wikman entitled "Automatic Data Treatment" describes in outline the application of computers to SEC data-handling, both from the point of view of interfacing and data-handling.

The final chapter by Pokorný covers the much more neglected area of "Precision and Accuracy of Results," and includes sections on instrumental errors, errors due to experimental conditions and variables, errors introduced by interpretaton of experimental data and on short- and long-term reproducibility of results.

All chapters give comprehensive references both to key published work as well as to work of a more exploratory investigatory nature. The high standard maintained throughout, together with the depth of coverage, makes this a book that the serious steric exclusion chromatographer cannot afford to be without.

B. K. Tidd

Topics in Enzyme and Fermentation Biotechnology, Volume 9

Edited by Alan Wiseman. Pp. 217. Ellis Horwood. 1984. Price £21. ISBN 0 85312 633 X (Ellis Horwood); 0 470 20031 6 (Halstead Press); ISSN 0140 0835.

"Topics in Enzyme and Fermentation Biotechnology" has appeared annually since 1977, but Volume 9 is the second volume to be published this year. Earlier volumes have covered a wide range of topics, including medicinal uses of enzymes, enzyme recovery, antibiotics and industrially important microorganisms. Similar diversity is found in this volume.

Boulton and Ratledge have reviewed the physiology of microorganisms that use the higher, linear alkanes. The metabolism of related compounds such as alkynes and carboxylic acids is also mentioned, especially when these are formed by the oxidation of alkanes. For the microbiologist, there is an interesting section on the effect of alkanes on cell morphology.

Lowe has contributed the longest chapter, on the application of reactive dyes, mainly of the triazine type, to biotechnology. Naturally, the use of immobilised dyes for affinity chromatography of proteins is given prominence. Techniques for preparing dye conjugates and carrying out the adsorption and elution of proteins are described. A large table giving literature references to successful methods for the isolation of a large number of proteins would have been even more useful if the type of dye used had been included. Less well known techniques such as affinity electrophoresis and the separation of proteins by distribution between two liquid phases, one of which contains a conjugate of a triazine dye with a water-soluble polymer such as polyethylene glycol, are included. This chapter ends with a discussion of the mode of interaction between proteins and dyes. Another useful table provides information about the inhibition of a range of enzymes by triazine dyes.

A chapter by Bickerstaff on applications of immobilised enzymes to fundamental studies on enzyme structure and function, as its title suggests, is not directly concerned with the applications of molecular enzymology to biotechnology but rather the converse. Immobilised enzymes not only provide a useful model of membrane-bound cellular enzymes, but also permit studies of enzyme denaturation and renaturation and the effect of a designed micro-environment on enzyme activity. In addition, by immobilising a sub-unit of a polymeric enzyme, the details of sub-unit interactions can be investigated conveniently.

The final chapter, by the Editor, on the progress with design of enzymes and mimics, is brief and provocative. The relative merits of chemical synthesis, chemical modification and genetic engineering are assessed. Some examples of non-peptide enzyme models are also given.

The price and wide range of topics covered by this and preceding volumes probably limit the potential market to libraries. Any institution involved in teaching or research in biotechnology will find this volume a valuable addition to the proliferating literature in the field.

D. T. Elmore

Open Tubular Column Gas Chromatography: Theory and Practice

Milton L. Lee, Frank J. Yang and Keith D. Bartle. Pp. xii + 445. Wiley-Interscience. 1984. Price £45.60. ISBN 0 471 88024 8.

Within the last few years several monographs and practical guides have been published on capillary GC but nobody has attempted to write a comprehensive text-book on the subject. This book aims to bring together the relevant theory, state-of-the-art instrumentation and applications of capillary GC and its authors are all accomplished practitioners of the technique.

The chromatographic theory section covers about 30 pages. It is concise, readable and well illustrated although the use of non-standard symbols leads to some confusion. This chapter whets the appetite about the theoretical advantages of microbore capillary columns (i.d. <50 μm) for highest efficiency, although the very high inlet pressures required mean that they are impractical in most modern GCs.

The chapter on column technology is comprehensive and excellently balanced. It covers the surface chemistry of fused silica, deactivation, stationary phase chemistry and the coating and testing of capillary columns. In the next chapter aspects of instrumentation are discussed and there are some good illustrations of injectors and various types of detectors and their principles of operation.

There is good coverage of the practical aspects of capillary GC and the listings of the pros and cons of different injection methods (split, splitless, direct and on-column) is particularly useful.

The second half of the book is devoted to applications and is divided by chemical class and sample type. While the first half is very readable this part is more encyclopaedic in its approach. It includes many well chosen chromatograms covering examples ranging from extraordinary temperature related inversions of retention times on liquid crystal phases to multi-dimensional separation of complex mixtures. There are some good examples of applications in the analysis of biological samples.

Whilst succeeding in most of its aims the book has some limitations. The treatment of the electron-capture detector is inadequate. There is no mention of the temperature dependence of the response and the standard text-book on the subject by Poole is not mentioned. Multi-dimensional chromatography is poorly covered with an over-complex example and confusing terminology with "valveless" switching being referred to as "valve" switching. However, I think that the most fundamental weakness of the book is that only six pages are devoted to quantitative analysis and this mostly relates to methods of standardisation. As quantitative limitations are the mains reasons why capillary columns have still not replaced packed columns for many applications a detailed discussion of how to achieve good quantitative results and what can be expected with different injection techniques should have been included.

On balance I think that this is a very worthwhile reference book for both novice and experienced users of capillary GC; with a fairly good index and over 1500 references it must be the best way into the published literature on capillary GC.

B. H. Woollen

Plastics Analysis Guide. Chemical and Instrumental Methods

A. Krause, A. Lange and M. Ezrin. Pp. xii + 358. Hanser (distributed in USA and Canada by Macmillan). 1984. Price DM118. ISBN 3 446 13587 1 (Hanser); 0 02 949410 9 (Macmillan).

This "Plastics Analysis Guide" is issued by the original publisher of Hummel, the standard work on the infrared spectra of polymeric materials.

It is divided into two sections: Part I (219 pp.) deals with chemical analysis and comprises a general classification of plastics, the detection and determination of characteristic elements, separation procedures for qualitative analysis, qualitative and quantitative methods of determination for individual materials, additives and standard test methods. Part II (65 pp.) deals with instrumental analysis, covering elemental analysis, separations based on solubility properties and discussion of the techniques of: infrared, ultraviolet, visible, nuclear magnetic resonance and mass spectroscopy; gas, liquid and thin-layer chromatography (including combined chromatographic - spectroscopic techniques); thermogravimetric, thermomechanical, differential thermal analysis and differential scanning calorimetry. A section on applications to the analysis of plastics gives practical and helpful details on the preparation of samples.

Lists are provided of standard test methods, including some titles of German Standards (DIN) and long lists of titles of US (ASTM) Standards. As the volume is the result of combined German and American work one would not expect necessarily to see the titles of appropriate British Standards included; however, although the equivalent numbers of some International Standards are given, more of these could well have been listed in the appropriate section.

There are some 120 numbered references and 16 general references for Part I and a total of 117 references to Part II, covering collections of spectra, books, technical literature and American analytical service laboratories, manufacturers and suppliers. The book has a general index, an index of authors, an index of infrared spectra (84 of which are collected together at the end of Part II) and an index to the figures and tables given in the text.

Even for the analytical scientist who is experienced in the field of plastics and polymers the Guide offers an up-to-date collection of data, and for the newcomer to the field it provides a splendid introduction to the various approaches that may be made to the qualitative and quantitative detection, identification and determination of polymers, with their additives in formulations.

The principal criticism perhaps would be that although the section which covers chemical methods will be of of considerable assistance to those without access to more sophisticated equipment, the section devoted to instrumental methods does not go into much depth with regard to the various techniques. It may be hoped that at some time these authors will bring out a book about the instrumental methods alone, where much detail could then be given for each.

The present Guide, nevertheless, is recommended strongly for the libraries of both academic and industrial establishments (and perhaps more than one copy will be needed so as to avoid a waiting list of borrowers, at least until the next edition of the well-known British work on the same topic comes along).

D. Simpson

Kinetics and Mechanisms of Electrode Processes

Edited by Brian E. Conway, J. O'M. Bockris, Ernest Yeager, S. U. M. Khan and Ralph E. White. *Comprehensive Treatise of Electrochemistry, Volume 7.* Pp. xviii + 788. Plenum. 1983. Price $95. ISBN 0 306 41152 0.

The Editors in their Preface to Volume 7 call electrode kinetics, "the heart of electrochemistry." This is axiomatic and, if mechanism may be assigned to the brain, makes this the most important volume of the series. It stands, indeed, on its own as the most important recent text on the core of electroanalytical chemistry. Except for the individual authors' own publications, the literature coverage rarely extends beyond 1977–1978. The reason for this is to be found in the general preface to the series wherein the objective is defined as, "an attempt at making a mature statement about the present position in the vast area of what is best looked at as a new interdisciplinary field," without making "each article emphasise the most recent situation." A distillation of the thoughts and experiments of the past century and a half and particularly of the last 40 years is to be expected, and where controversy still exists a clear presentation of opposing views.

The coverage and the authority with which it is presented are best defined by listing the ten sections. The plunge *in medias res* of quantum mechanical interpretation is made by Dogonadze and Kusnetsov on the continuum theory and Khan and Bockris on molecular aspects, followed by Krishtalik on kinetics at metal - solution interfaces, Appleby on electrocatalysis, Enyo on the hydrogen reaction at electrocatalytically active metals and Tarasevich, Sadkowski and Yeager on oxygen electrochemistry. Deposition and dissolution of metals and alloys is divided into electrocrystallisation by Budevski and mechanisms, kinetics, texture and morphology by Despić.

Processes at semi-conductor electrodes are dealt with by Memming and molten salt media by Inman and Lovering. The longest chapter is on organic electrode processes by Rudd and Conway.

Obviously basic matters of controversy are covered. As yet there is no agreement on the nature of the activation processes, for example. In this and other aspects there are important schools of alternative, indeed conflicting, thought. The Editors have been at pains to secure clear and fair expositions of alternative views in such areas, as is obvious from the listing of topics and authors, in order that practitioners may have the current situation set out plainly before them and may be stimulated to seek a resolution of the several matters. Experience, and meditation about the meaning, of the field is a prerequisite for appreciation of the disquisition here set forth. The density and elaboration of mathematical description and treatment varies somewhat, but is always considerable and needs practical mathematical experience to cope with and appreciate. Skipping the maths is to deny the virtues of the text, which brings alternative views into contact within the covers of one book, and saves to a considerable extent the study of a large number of texts from individual schools. It would be invidious to single out one or two chapters for comment: proper appraisal of the whole would fill many pages. Suffice to say that close and enduring study is necessary and will amply repay the time expended. The price is high, but the book is nevertheless good value for money, and should be in the collection of any serious student of the field.

E. Bishop

Analyse des Réactions Chimiques en Solution

J.-Y. Gal and J. Gal. Pp. viii + 408. Dunod. 1983. Price F120. ISBN 2 04 015579 1.

Teaching equilibria in solutions at a high level is well known to be a difficult task and improvements have been gained by the use of computers. It is therefore not easy to understand the aim of the authors. In their introduction they indicate their proposal to use conditional constants according to Schwarzenbach and Ringbom and that modern computers will allow students to conduct rigourous calculations without approximations. However, no programs are given and the results developed throughout the book do not seem to have involved the use of computer facilities.

Many non-rigorous treatments are to be found all through this book: the influence of the numerical value of the acidity constant on the dissociation of acids and bases by dilution is ignored and only the classical (and false) Ostwald treatment is developed. The well known relationship $\mathrm{pH} = \frac{1}{2}(\mathrm{p}k_2 + \mathrm{p}k_1)$ is not given with its validity domain ($\mathrm{p}k_2 + \mathrm{p}k_1 \approx \mathrm{p}K_i$). The exercise on the titration of phosphoric acid by sodium hydroxide in the presence of silver ions (pp. 353–358) is treated without taking into consideration many subtleties of this interesting titration (for instance, the transformation of Ag_3PO_4 in AgOH before the titration of the third H_3PO_4 acidity).

In conclusion, this book does not contain any new treatment compared with classical textbooks on solution chemistry and cannot be recommended.

R. Rosset

Identification and Analysis of Organic Pollutants in Air

Edited by Lawrence H. Keith. Pp. xxii + 486. Ann Arbor Science. 1984. Price £55. ISBN 0 250 40575 X.

In spite of the title, this is not a textbook but a compilation of 28 papers presented at an American Chemical Society Symposium of the same title, given at Kansas City in September, 1982.

The papers are a reasonably balanced mixture, but most are concerned with environmental air contaminants and again, in spite of the title, many are about sample collection, with two papers being basic studies of porous polymer adsorbents. Only four of the papers cover workplace exposure monitoring, although a few others apply to both the environmental and workplace aspects of airborne contaminants. Six papers are of the review type and the others are in general good, up-to-date technical papers containing a good deal of useful information and data.

The most popular subject is, not surprisingly, polycyclic aromatic hydrocarbons, with twelve papers including this topic, using a variety of trapping techniques, clean-up procedures and analysis by GC, MS, HPLC, voltammetry and luminescence spectroscopy. There are six papers on emissions from combustion processes and the remainder cover monitoring for a variety of substances, including monoterpenes, dialkyl sulphates, phthalate esters, pesticides and gasoline.

As a compilation of symposium papers this book has many papers useful to the environmental chemist. It is, however, rather expensive considering that it is not a textbook.

David T. Coker

Instrumental Liquid Chromatography. A Practical Manual on High-Performance Liquid Chromatographic Methods. Second Completely Revised Edition

N. A. Parris. *Journal of Chromatography Library, Volume 27.* Pp. xiv + 432. Elsevier. 1984. Price $86.50; Dfl225. ISBN 0 444 42061 4 (Volume 27); 0 444 41616 1 (Series).

The appearance of a second edition of any book is a sure sign that the first edition was sufficiently well received to warrant the effort and expense of a major revision. Certainly over the 7 years since this book's first appearance, the art of instrumental liquid chromatography has changed enormously. Perhaps this is why the publishers chose to emphasise "Second, Completely Revised" on the cover. Certainly much as been changed and updated, resulting in a well written text that will doubtless find its way into many laboratory collections. Complete coverage of topics in books of this type is difficult to ensure but it is disappointing that Chapter 13, on establishing the identity of a peak, only gives three lines to diode array spectrometry, for example. A surprising omission is a comprehensive discussion of the role of computers in the control of liquid chromatography. The seven pages devoted to Chapter 6, "Modern Electronic Technology and Its Impact on LC Automation," in no way reflects the importance and size of this topic.

The book is well produced and substantially free from typographical errors. Not withstanding its shortcomings, I am sure that it will become a standard work for both newcomers to the field and experts alike.

C. Burgess

INSTRUCTIONS TO AUTHORS

The Analyst publishes papers on all aspects of the theory and practice of analytical chemistry, fundamental and applied, inorganic and organic, including chemical, physical and biological methods. Papers may be submitted for publication by members of The Royal Society of Chemistry or by non-members. There is no page charge for papers published in *The Analyst.*

The following types of papers will be considered.

Full papers, describing original work.

Short papers, also describing original work, but shorter and of limited breadth of subject matter; there will be no difference in the quality of the work described in full and short papers.

Communications, which 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. Communications will normally be examined by one referee.

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Every paper (except Communications) will be submitted to at least two referees, by whose advice the Editorial Board of *The Analyst* will be guided as to its acceptance or rejection. Papers that are accepted must not be published elsewhere except by permission. Submission of a manuscript will be regarded as an undertaking that the same material is not being considered for publication by another journal.

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Regional Advisory Editors. For the benefit of potential contributors outside the United Kingdom, a Panel of Regional Advisory Editors exists. Requests for help or advice on any matter related to the preparation of papers and their submission for publication in *The Analyst* can be sent to the nearest member of the Panel. Currently serving Regional Advisory Editors are listed in each issue of *The Analyst.*

Manuscripts. Papers should be typewritten in double spacing on one side *only* of the paper. Three copies of text and illustrations should be sent to the **Editor, The Analyst, The Royal Society of Chemistry, Burlington House, London, W1V 0BN,** and a further copy retained by the author.

Proofs. The address to which proofs are to be sent should accompany the paper. Proofs should be carefully checked and returned immediately (by Air Mail from outside Europe).

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Notes on the Writing of Papers for *The Analyst*

Manuscripts should be in accordance with the style and usage shown in recent copies of *The Analyst.* Conciseness of expression should be aimed at: clarity is increased by adopting a logical order of presentation, with suitable paragraph or section headings.

To facilitate abstracting and indexing by Chemical Abstracts Service, and other abstracting organistions, it would be helpful if at least one forename could be included with each authors family name.

Descriptions of new methods should be supported by experimental results showing accuracy, precision and selectivity.

The recommended order of presentation is as indicated below:

(*a*) *Title.* This should be as brief as is consistent with an adequate indication of the original features of the work. The analytical method used in the work should be mentioned in the title.

(*b*) *Synopsis.* A synopsis of about 100 words, giving the salient features and drawing attention to the novel aspects, should be provided for all papers.

(*c*) *Keywords.* Up to 5 keywords, indicating the topics of importance in the work described, should be included after the synopsis.

(*d*) *Aim of investigation.* An introductory statement of the object of the investigation with any essential historical background, followed, if necessary, by a *brief* account of preliminary experimental work.

(*e*) *Description of the experimental procedures.* Working details must be given concisely. Analytical procedures should preferably be given in the form of instructions; well known operations should not be described in detail.

(*f*) *Results.* These are best presented in tabular form, followed by any statistical evaluation, which should be in accordance with accepted practice.

(*g*) *Discussion of results.* This section will comment on the scope of the method and its validity, followed by a statement of any conclusions drawn from the work.

Nomenclature. Current internationally recognised (IUPAC) chemical nomenclature should be used. Common trivial names may be used, but should first be defined in terms of IUPAC nomenclature.

SI units. The SI system of units should be used. These units are summarised in the Appendix. The effect on current style of papers for *The Analyst* includes the following:

(*a*) dimensions should preferably be given in metres (m) or in millimetres (mm);

(*b*) temperatures should be expressed in K or °C (not °F);

(*c*) wavelengths should be expressed in nanometres (nm) (not mμ);

(*d*) frequency should be expressed in Hz (or kHz, etc.), not in c/s or c.p.s.; rotational frequency can be denoted by use of s^{-1};

(*e*) radionuclide activity will be expressed in becquerels (Bq) or curies (Ci); $1\ Ci = 3.7 \times 10^{10}\ Bq$;

(*f*) the micron (μ) will not be used; 10^{-6} m will be 1 μm.

Abbreviations. SI units should be used. Normality and molarity are generally expressed as decimal fractions (*e.g.*, 0.02 N, 0.375 M). Abbreviational full stops are omitted after the common contractions of metric units (*e.g.*, ml, g, µg, mm) and other units represented by symbols. Abbreviations other than those of recognised units should be used sparingly in the text.

Percentage concentrations of solutions should be stated in internationally recognised terms. Thus the symbols "*m*" for mass and "*V*" for volume are to be used instead of "w" for weight and "v" for volume. The following show the manner of expressing these percentages together with an acceptable alternative given in parentheses: % *m*/*m* (g per 100 g); % *m*/*V* (g per 100 ml); % *V*/*V*. Further implications of the use of the term "mass" are that "relative atomic mass" of an element (A_r) replaces atomic weight, and "relative molecular mass" of a substance (M_r) replaces molecular weight.

Concentrations of solutions of the common acids are often conveniently given as dilutions of the concentrated acids, such as "dilute hydrochloric acid (1 + 4)," which signifies 1 volume of the concentrated acid mixed with 4 volumes of water. This avoids the ambiguity of 1 : 4, which might represent either 1 + 4 or 1 + 3. Dilutions of other solutions can be expressed in a similar manner.

Tables and diagrams. The number of tables should be kept to a minimum. Column headings should be brief. Tables consisting of only two columns can often be arranged horizontally. Tables must be supplied with titles and be so set out as to be understandable without reference to the text.

Either tables or graphs may be used but not both for the same set of results, unless important additional information is given by so doing. The information given by a straight-line calibration graph can usually be conveyed adequately as an equation or statement in the text.

The style used in headings to tables and in labels on the axes of graphs, where the numbers represent numerical values, is, for example: Volume/ml. The diagonal lines (solidus) will not be used to represent "per." In accordance with the SI system, units such as grams per millilitre are already expressed in the form g ml^{-1}. For a table (or graph), this would appear as: Concentration of solution/g ml^{-1}. It should be noted that the "combined" unit, g ml^{-1}, must not have any "intrusive" numbers. To express concentration in grams per 100 millilitres, the word "per" will still be required: Concentration/g per 100 ml. It may be preferable for an author to express concentrations in grams per litre (g l^{-1}) rather than grams per 100 ml.

Most diagrams will be retraced and lettered in order to achieve uniform line thicknesses and lettering size and style, so it is not essential to prepare specially traced drawings. However, all diagrams should be carefully and clearly drawn on good quality paper and should be clearly lettered. If possible, complicated flow charts, circuit diagrams, etc., should be supplied as artwork for direct reproduction in order to avoid time-consuming and expensive redrawing.

Three sets of illustrations should be provided, two sets of which may be made by any convenient copying process for transmission to the referees.

All diagrams should be accompanied by a separately typed set of captions. Wherever possible, extensive identifying lettering should be placed in the caption rather than on lines on graphs, etc.

Photographs. Photographs should be submitted only if they convey essential information that cannot be shown in any other way. They should be submitted as glossy or matt prints made to give the maximum detail. Colour photographs will be accepted only when a black-and-white photograph fails to show some vital feature and can be supplied either as prints or transparencies.

References. References should be numbered serially in the text by means of superscript figures, *e.g.*, Foote and Delves,[1] Burns *et al.*[2] or Hirozawa,[3] and collected in numerical order under "References" at the end of the paper. They should be listed, with the authors' initials, in the following form (double-spaced typing):

1. Foote, J. W., and Delves, H. T., *Analyst*, 1983, **108**, 492.
2. Burns, D. T., Glocking, F., and Harriott, M., *J. Chromatogr.*, 1980, **200**, 305.
3. Hirozawa, S. T., *in* Kolthoff, I. M., and Elving, P. J., *Editors*, "Treatise on Analytical Chemistry," Part II, Volume 14, Wiley, New York, 1971, p. 23.

Journal titles should be abbreviated according to the *Chemical Abstracts Service Source Index (CASSI)*.

For books, the edition (if not the first), the publisher and the place and date of publication should be given, followed by the page number.

Authors must, in their own interest, check their lists of references against the original papers; second-hand references are a frequent source of error. The number of references must be kept to a minimum.

Appendix

The SI System of Units

In the SI system there are seven base units—

Physical quantity	*Name of unit*	*Symbol for unit*
length	metre	m
mass	kilogram	kg
time	second	s
electric current	ampere	A
thermodynamic temperature	kelvin	K
amount of substance	mole	mol
luminous intensity	candela	cd

There are two supplementary dimensionless units for plane angle (radian, rad) and solid angle (steradian, sr). Some derived SI units that have special names are as follows—

Physical quantity	*Name of unit*	*Symbol for unit*	*Definition of unit*
energy	joule	J	$\mathrm{kg\,m^{2}\,s^{-2}}$
force	newton	N	$\mathrm{kg\,m\,s^{-2} = J\,m^{-1}}$
power	watt	W	$\mathrm{kg\,m^{2}\,s^{-3} = J\,s^{-1}}$
electric charge	coulomb	C	$\mathrm{A\,s}$
electric potential difference	volt	V	$\mathrm{kg\,m^{2}\,s^{-3}\,A^{-1} = J\,A^{-1}\,s^{-1}}$
electric resistance	ohm	Ω	$\mathrm{kg\,m^{2}\,s^{-3}\,A^{-2} = V\,A^{-1}}$
electric capacitance	farad	F	$\mathrm{A^{2}\,s^{4}\,kg^{-1}\,m^{-2} = A\,s\,V^{-1}}$
frequency	hertz	Hz	$\mathrm{s^{-1}}$
magnetic flux density (magnetic induction)	tesla	T	$\mathrm{kg\,s^{-2}\,A^{-1} = V\,s\,m^{-2}}$
radionuclide activity	becquerel	Bq	$\mathrm{s^{-1}}$

Examples of other derived SI units are—

Physical quantity	*SI unit*	*Symbol for unit*
area	square metre	$\mathrm{m^{2}}$
volume	cubic metre	$\mathrm{m^{3}}$
density	kilogram per cubic metre	$\mathrm{kg\,m^{-3}}$
velocity	metre per second	$\mathrm{m\,s^{-1}}$
angular velocity	radian per second	$\mathrm{rad\,s^{-1}}$
acceleration	metre per second squared	$\mathrm{m\,s^{-2}}$
magnetic field strength	ampere per metre	$\mathrm{A\,m^{-1}}$

Certain units will be allowed in conjunction with the SI system, *e.g.*—

Physical quantity	*Name of unit*	*Symbol for unit*	*Definition of unit*
volume	litre	l	$\mathrm{10^{-3}\,m^{3} = dm^{3}}$
magnetic flux density (magnetic induction)	gauss	G	$\mathrm{10^{-4}\,T}$
temperature, t	degree Celsius	°C	$t/\mathrm{°C} = T/\mathrm{K} - 273.16$
radionuclide activity	curie	Ci	$\mathrm{3.7 \times 10^{10}\,Bq}$
energy	electronvolt	eV	$\mathrm{1.6021 \times 10^{-19}\,J}$

The common units of time (*e.g.*, minute, hour, day) and the angular degree (°) will continue to be used in appropriate contexts.

Decimal multiples and submultiples have the following names and symbols (for use as prefixes)—

10^{-3}	milli	m	10^{3}	kilo	k
10^{-6}	micro	μ	10^{6}	mega	M
10^{-9}	nano	n	10^{9}	giga	G
10^{-12}	pico	p	10^{12}	tera	T
			10^{15}	peta	P
			10^{18}	exa	E

Compound prefixes (*e.g.*, mμm) should not be used; $10^{-9}\ \mathrm{m} = 1\ \mathrm{nm}$.

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Over many years international organizations, national organizations and private concerns have prepared standardized methods of analysis for food products, including milk and milk products, for purposes of quality control, assessment of nutritive content, enforcement of legal requirements and affirmation of safety. This activity is concerned with identifying the most appropriate current methodology and codifying it in authoritative documents.

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A201 for further information. See page iv

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