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APPLICATIONS OF FABRY—PÉROT INTERFEROMETRY IN MULTI-ELEMENT FLAME EMISSION ANALYSIS

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(Received 22nd February 1978)

SUMMARY

A system for multi-element flame emission analysis based on a scanning Fabry—Pérot interferometer is described and evaluated. Detection limits and linearity of response for ten elements commonly determined by flame photometry compare well with conventional single-element methods. Analyses for several elements in tap water, serum, urine, standard orchard leaf and low-alloy steel demonstrate the good accuracy and precision of the technique; the resolution allows up to five elements to be determined simultaneously.

The determination of trace elements in a wide variety of matrices has been a long-standing problem in chemical analysis. The need for new or improved analytical procedures increases with the development of new energy sources, the concern over the environmental impact of pollution, and the advance in clinical diagnostic techniques. In atomic emission spectrometry, multi-element analysis is possible with photographic plates, and with rapid scan spectrometers or direct-reading multichannel detectors, which have the advantages of speed, large working range and quantitative accuracy. Rapid scanning techniques have been reviewed recently [1]; most of them suffer from problems associated with irreproducibility because of the nature of mechanical translation. Direct-reading detector arrays [2] become complex and costly as the number of channels increases.

The Fabry—Pérot interferometer is an interesting wavelength dispersing element; it provides high resolution with good light throughput but suffers from overlapping spectral orders. In a preliminary investigation a scanning F—P interferometer was proposed [3] for multi-element atomic emission analysis; if the proper design parameters are used, up to ten elements can be determined simultaneously on the millisecond time scale with sensitivities comparable with single-channel flame emission spectrometers. The theory [3] will not be reproduced here. Briefly, scanning of over one free spectral range [4] allows each and every atomic emission line to be transmitted, most likely at a different point during the scan and typically at different spectral orders of the interferometer. As long as the total number of emission

lines is small, they should be resolved so that simultaneous determinations are possible. Many practical analytical situations are restricted to only three to five elements, and this analytical scheme with the interferometer seems best-suited for these.

This paper describes a second-generation Fabry—Pérot atomic emission spectrometer; the performance of the initial interferometer [3] and the scope of application are greatly increased. The performance for both single-element and multi-element analysis for ten common elements in matrices such as tap water, serum, urine, orchard leaf, and low alloy steel is evaluated critically.

EXPERIMENTAL

Optics

Significant improvement in resolution and throughput over the original design [3] is achieved by the optical arrangement shown in Fig. 1. The main differences are: the *f*-number of the collection lens, L1, is much more favorable than before; the enlarged pinhole allows sixteen times more light to pass and a larger fraction of the flame is imaged; light entering the interferometer is convergent so that alignment becomes simpler. The F—P interferometer used is a Tropel Model CL-100 system with mirrors coated for the visible region. The mirror spacing determines [3] whether a particular group of atomic lines is resolved or not. The spacing is typically between 10 μm and 20 μm. The finesse [3] of the system is always better than 20 with proper alignment of all the optical components.

Electronics

A schematic diagram of the scanning and the detection electronics is shown in Fig. 2. Scanning is accomplished with a ramp generator and a high voltage amplifier [5]. Atomic emission is detected by a RCA IP-21 photomultiplier tube, powered by a Hamner NV-13 high-voltage supply. A Datel Model AM200A instrumentation amplifier acts as the buffer for the phototube output. A 6-channel sample-and-hold circuit [5], based on Datel SHM-LM-2 modules and National Semiconductor LM311 voltage comparators, allows signal peaks to be sampled at any six pre-determined points along the

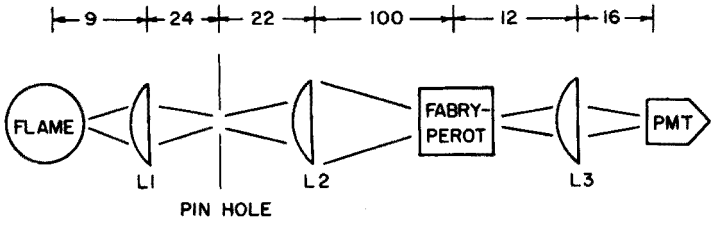

Fig. 1. Optical arrangement for F—P emission analysis (all spacings in cm). L1, aspherical condenser lens, 68-mm *f*.l., 60-mm diameter; L2, 200-mm *f*.l., 130-mm diameter; L3, 150-mm *f*.l., 105-mm diameter; pinhole, 4-mm diameter.

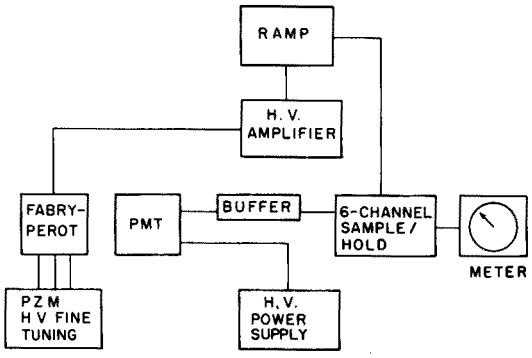

Fig. 2. Block diagram of electronic components. See text for details.

scan and read off on a panel meter up to 3 min after the scan. The unit is self-contained and an oscilloscope becomes unnecessary. In studies involving signal averaging, the sample-and-hold circuit is replaced by a Digital Equipment Corporation PDP/11-10 minicomputer with a Laboratory Peripheral System.

Burner—nebulizer

A Beckman Model 4030 total-consumption burner with a Model 9220 regulator is used. Flow rates (Brooks Model 1355-OOAIFAA rotameter) are 8.2 l min^{-1} for hydrogen and 2.5 l min^{-1} for oxygen, corresponding to pressures of 1.5 and 8.5 psi, respectively. The solution consumption rate is 0.72 ml min^{-1} to produce a slightly reducing flame.

Samples

Standard solutions are prepared with reagent-grade salts and deionized water. Samples of tap water were run without pretreatment other than the addition of a lithium internal standard. Urine samples, collected from five different subjects, were refrigerated until analysis. Serum samples (dehydrated human blood serum, Dade Division, American Hospital Supply Company) were reconstituted in 5 ml of deionized water. A portion (5 ml) of each urine or serum sample was prepared for analysis by adding 25 mg of Li and 25 ml of 2% sterox (a surfactant to prevent clogging of the nebulizer), and diluting to 250 ml with deionized water.

NBS SRM 1571 orchard leaves were analyzed with minimum sample preparation. Samples (1 g) of dry orchard leaves, dissolved in 50 ml of HNO_3—$HClO_4$ solution (3 : 2), were diluted to 500 ml. A blank containing the acid mixture and deionized water was used. Steel samples were prepared from NBS SRM 163 powdered low alloy chromium steel; portions (1 g) of the alloy were dissolved in 50 ml of concentrated nitric acid, 60 mg of Sr was added as internal standard and the solution was diluted to 500 ml. No silica or graphite was present after dissolution and thus filtration was not necessary prior to analysis.

RESULTS AND DISCUSSION

Detection limits and working range

Table 1 presents the detection limits observed (defined for the point where $S/N = 3$) for the ten elements studied. For sodium, 0.06 $\mu g\ ml^{-1}$ was present in the deionized water as determined by standard addition, so that the values quoted are extrapolated estimates. Absolute detection limits were calculated from the relative detection limits by taking into account the total time for analysis and the solution consumption rate. The signal-averaged values result from numerically averaging 1,000 scans of the F—P interferometer by the computer to decrease the noise level. The total time for data accumulation is then 3.5 s. Similar values are obtained if the averaging is performed with the sample-and-hold circuit.

Generally, the single-scan relative detection limits are much better than those reported earlier [3]. Although they are an order of magnitude poorer than those for single-element flame emission [6], the use of single scan is restricted frequently to situations where the absolute detection limits are important. In conjunction with pulsed atom sources, impressive results can be obtained. Relative detection limits for signal-averaged multiple scans compare favorably with other flame emission methods [6]. Not all elements show greatly improved detection limits on signal averaging; in some cases the background noise results from molecular emission and is not completely random.

Calibration plots of signal vs. concentration are shown in Fig. 3. All of the elements display linearity over a minimum of three orders of magnitude in concentration, with sodium providing a linear response from 0.06 (residual concentration) to 1000 $\mu g\ ml^{-1}$. The relatively small flame serves as a sufficiently thin excitation source such that self-absorption effects were not observed for any element at concentrations below 1000 $\mu g\ ml^{-1}$. Peak height or peak area may be used, but the former is preferred because the probability of spectral interference is lower.

TABLE 1

Detection limits ($S/N = 3$)

Element	Wavelength (nm)	Single scan		Signal-averaged	
		Relative ($\mu g\ ml^{-1}$)	Absolute (ng)	Relative ($\mu g\ ml^{-1}$)	Absolute (ng)
Mn	403.1	0.25	0.076	0.065	2.7
K	404.4	0.75	0.22	0.024	1.0
Rb	420.2	1.0	0.30	0.060	2.5
Ca	422.7	0.10	0.031	0.019	0.82
Cr	425.4	1.2	0.36	0.22	9.4
In	451.1	0.075	0.022	0.012	0.50
Sr	460.7	0.79	0.24	0.16	6.9
Ba	553.6	3.0	0.90	0.55	24.
Na	589.0	0.009	0.0027	0.0007	0.03
Li	670.8	0.15	0.045	0.021	0.88

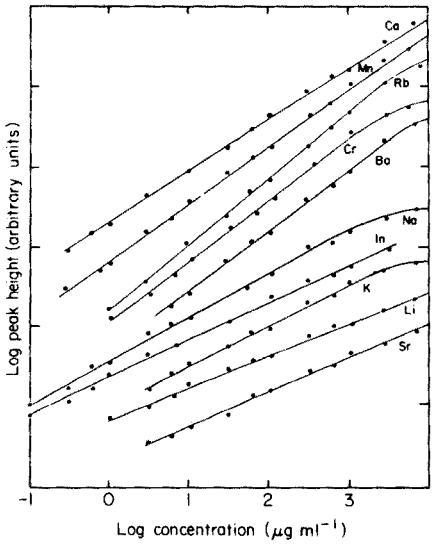

Fig. 3. Calibration plots for ten elements.

Accuracy and precision

In addition to the interference effects typical of flame atomic spectrometry [7], spectral interference from emission of molecular species may be serious in F—P interferometry because of the overlapping spectral orders. The interference effects of various anionic species present in matrices such as serum or urine are summarized in Table 2. Those elements not listed (Rb, In and Li) showed no appreciable effects with any of the five anions. The table shows the percentage reduction (—) or enhancement (+) of the emission signal for a solution (100 μg ml^{-1}) of the cation in the presence of a tenfold excess of the anion compared with the corresponding chloride salt. Overall, these values

TABLE 2

Chemical interference effects[a]

Anions	Change in signal (%)						
	Na	K	Sr	Ba	Ca	Cr	Mn
PO_4^{3-}	0	—0.4	—5	—8	—5	+0.1	+0.5
SO_4^{2-}	0	+0.5	—3	—4	—0.2	0	+2
CO_3^{2-}	—0.2	0	—0.5	—0.3	0	0	0
$CH_3CO_2^-$	0	+0.1	0	0	0	0	+1
NO_3^-	0	+1	0	0	+1	0	0

[a]Values are relative to the corresponding chloride salts for 100 μg ml^{-1} of the cation and 1000 μg ml^{-1} of the anion.

are typical of chemical interferences in solution. Of the three elements determined routinely in urine and serum (Na, K, Ca), only calcium exhibits appreciable interference. When the phosphate to calcium ratio is decreased to the levels present in such biological samples, only a 2% suppression in calcium emission is found. Thus, if the standard addition method is used these difficulties will be minimized.

The simultaneous determination of sodium and calcium in tap water is performed with a lithium internal standard. A Corning CS-1-64 filter in the optical path brings the signals from the two elements to approximately the same magnitude. For comparison, sodium was determined separately with a Beckman Model DU flame photometer and calcium by EDTA titration [8]. The results of this study are shown in Table 3; the precision of the method is comparable with conventional flame photometric methods but inferior to titrimetric methods.

The potential of the F—P interferometer method for the analysis of agricultural materials is best evaluated from the determination of K, Ca and Na in NBS standard orchard leaves; a Corning CS-3-77 filter helps to balance the emission signals. Although lithium is a trace component of orchard leaves, it was not detected under these conditions and was added as an internal standard. Overall, the precision (2—4%) and the accuracy (2—6%) of the results (Table 3) are comparable with or better than conventional single-element photometric methods for samples of similar origin [9].

Table 3 shows the results of simultaneous determinations of Cr and Mn in steel by the F—P method. A Corning CS-1-60 filter eliminated any residual sodium emission and a strontium internal standard was used. In general, the precision is slightly worse than flame emission analysis for single elements [10] but the accuracy is slightly better.

TABLE 3

Multi-element F—P analysis in various matrices

Matrix and element	F—P value		Reference[a] value	
	$\bar{x}$	σ_x	$\bar{x}$	σ_x
Tap water				
Na (mg l^{-1})	33.6	1.5	37.4	1.8
Ca (mg l^{-1})	100.2	2.6	96.52	0.35
Orchard leaves				
Ca (mg g^{-1})	19.6	0.6	20.9	0.3
K (mg g^{-1})	14.3	0.6	14.7	0.3
Na ($\mu g\ g^{-1}$)	80.6	1.3	82.0	3.0
Steel				
Mn (mg g^{-1})	8.48	0.19	8.97	—
Cr (mg g^{-1})	9.36	0.20	9.82	—

[a]Reference from NBS certificate or an alternative method of analysis. See text for details.

TABLE 4

Analysis of urine and serum

	Na (meq)		Ca (meq)		K (meq)			Na (meq l^{-1})		Ca (mg dl^{-1})		K (meq l^{-1})	
	A[a]	B[a]	A	B	A	B		A[a]	B[a]	A	B	A	B
Urine							*Serum*						
1	190	187	210	202	120	110	1	140	149	10	9.9	2.5	2.7
2	110	115	120	115	90	92	2	143	149	8.2	8.5	5.2	4.9
3	170	175	130	120	50	55	3	130	127	11	11.5	3.1	3.6
4	180	184	180	175	100	96	4	105	112	8.5	8.1	3.7	4.0
5	120	130	95	90	130	127	5	138	140	9.2	9.6	4.2	4.5

[a]F—P values are shown in columns A with reference values (flame photometry for urine and assay [11] for serum) in columns B.

Results for the simultaneous determination of Na, K and Ca in urine and blood serum, with Li as internal standard, are presented in Table 4. A Corning CS-1-60 filter was used to attenuate the sodium emission. Generally, accuracy within 7% of the assay values [11] was obtained for the serum samples. The results for urine specimens agreed closely with those given by a Beckman DU flame photometer. Precision for both types of samples was usually less than 3%, which equals or improves on that given by commercial clinical multi-element flame photometers [12].

Conclusions

The Fabry—Pérot interferometer—flame emission technique preserves the sensitivity, accuracy and precision of conventional flame photometers while adding the capability for multi-element analysis for 3—10 elements. For diverse matrices such as tap water, urine, blood serum, orchard leaves and steel, spectral interference from molecular emission does not present a problem, and the simultaneous determination of typical elemental components in each is possible.

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DETERMINATION OF MINOR ELEMENTS IN STEEL BY CARBON FURNACE ATOMIC EMISSION SPECTROMETRY

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SUMMARY

Procedures are described for the determination of chromium, copper, manganese, nickel and soluble aluminium in steels by carbon-furnace atomic emission spectrometry. A single set of atomization conditions is optimum for all five elements but different dilutions of the sample solution are recommended for different analyte concentrations in the steels. Interferences from iron and nitric acid are small or insignificant and easily compensated.

Although the analytical application of the measurement of atomic emission signals during carbon-furnace atomization was first proposed in 1975 [1], and developments have been described which improve either the sensitivity [2, 3] or the range of elements [3, 4] to which the technique may sensibly be applied, only a few applications to real samples have so far been reported [2, 5, 6]. As an atomic emission source, the attraction of the carbon furnace lies in its relatively low operating temperature and simple spectral background. The low temperature should ensure that only lines of low excitation potential are observed, thus reducing the incidence and importance of spectral interference from line overlap when a matrix such as steel is atomized. The background signal is generated by the black-body radiation from the carbon tube, but if the tube is correctly designed and matched to the optical system of the spectrometer, the continuum background is reduced to Rayleigh scattering of tube wall radiation by species in the vapour phase [7], which can easily be compensated by background correction systems based on wavelength modulation [2, 6]. CN emission can be observed [8] if nitrogen is used as the purge gas but this is insignificant in an argon atmosphere. Weak oxide emissions have been observed during the atomization of calcium, strontium and magnesium [8] but these are not relevant to steel analysis. Ionization occurs during carbon-furnace atomization but can easily be suppressed with the usual ionization buffers [9].

Such freedom from spectral interferences suggests that carbon-furnace atomic emission spectrometry would be very suitable for the analysis of steels for a wide range of (but not all) elements, provided that there is no

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significant chemical effect of iron on the atomization of minor and trace elements. Although chemical interferences can be observed readily in carbon-furnace atomization when chloride solutions are applied, such problems are not observed in all media. For example, the use of nitric acid solutions frequently reduces such effects to a negligible level whether atomic absorption or emission is used for the analytical measurement [10]. This has been specifically demonstrated in carbon-furnace atomic emission for the determinations of lithium in copper [11] and barium in calcium carbonate rocks [6]. In the analysis of steel samples by carbon-furnace atomic absorption it was demonstrated that in a nitric acid medium, iron did not interfere in the determination of lead [12], vanadium or aluminium, and had relatively small effects on the determination of a number of other elements, at iron/analyte ratios corresponding to trace levels of the analytes in steels [13].

This paper demonstrates that carbon-furnace atomic emission can be used to determine a number of elements in steel, without chemical or other interference. The technique may be an attractive alternative to methods in current use.

EXPERIMENTAL

Apparatus

The instrument used for all measurements was a Perkin-Elmer 306 atomic absorption/emission spectrometer equipped with an HGA 72 heated graphite atomizer and coupled to a Servoscribe strip-chart recorder. The instrument was used in the emission mode and was set up and operated as described previously [1, 3].

Reagents

All solutions were prepared from analytical reagent-grade chemicals. Deionized water was used throughout. Stock solutions at an elemental concentration of 1000 μg ml^{-1} were prepared in 10^{-2} M nitric acid from aluminium sulphate, chromium(III) nitrate, copper(II) nitrate, manganese(II) nitrate and nickel(II) nitrate. These were diluted to 10 μg ml^{-1} for working stock solutions and were diluted further as required.

A stock 10 mg Fe ml^{-1} solution was prepared by dissolving 0.5 g of Specpure iron in 10 ml of 40% (v/v) nitric acid in a PTFE beaker and diluting to volume with water in a 50-ml volumetric flask.

Preparation of calibration solutions

Suitable aliquots of the stock iron solution and the working stock analyte solutions were taken to prepare the standard solutions shown in Table 1 appropriate to the concentration range of the analyte in each sample. In each case, iron and nitric acid were incorporated in the standards at concentrations equivalent to those in the diluted sample solution, and a blank was prepared with iron and nitric acid present at the appropriate level but with no added analyte.

TABLE 1

Preparation of standard and sample solutions for the determination of Cr, Cu, Mn, Ni and soluble Al in steels

Element	Range in steel (%)	Dilution of 1% sample soln.	Fe added to standards (μg ml^{-1})	Range of analyte in standards (μg ml^{-1})
Cr	0.02—0.05	10×	1000	0.1—0.5
	0.05—0.25	100×	100	0.05—0.25
Cu	0.01—0.06	10×	1000	0.2—0.8
Mn	0.1—0.5	100×	100	0.1—0.5
Ni	0.005—0.1	10×	1000	0.2—1.0
	0.05—0.25	50×	200	0.1—0.5
Al	0—0.05	10×	1000	0.1—0.5
	0.05—0.2	100×	100	0.05—0.2

Preparation of sample solutions

The sample (0.5 g) was dissolved in 10 ml of 40% (v/v) nitric acid in a PTFE beaker. The solution was filtered through a Whatman 542 filter paper and diluted to volume with water in a 50-ml volumetric flask. This solution was diluted to the required level, as shown in Table 1.

Instrument operation

The most sensitive atomic emission line for each element, viz., Cr, 425.43 nm; Cu 324.75 nm; Mn, 403.31 nm; Ni, 341.48 nm; Al, 396.15 nm, was used, at a spectral slit width of 0.07 nm (slit 2 on the PE 306). The instrument was used in the emission mode at × 1 scale expansion and signals were recorded at a chart speed of 1 cm s^{-1}. Graphite tubes modified by reducing the wall thickness at the centre [3] were used throughout and gave a maximum atomization temperature of 2880 K at the 999 digit setting. Aliquots (50 μl) were manually injected into the carbon furnace, dried at 373 K (HGA 72 setting 35) for 40 s and atomized under gas-stop conditions at 2880 K for 10 s for all elements. An argon gas flow of 1.5 l min^{-1} at 40 psi was admitted except during atomization. After each atomization, the maximum temperature button was depressed to ensure that no analyte or iron matrix was carried over to the next determination.

Aliquots of standard and sample solutions were injected sequentially into the carbon furnace and atomized. For each solution, the peak emission signal was recorded and the background tube emission signal (which was recorded separately at the same wavelength as the analyte) obtained at the time of peak analyte emission was subtracted to give the net analyte emission signal. The concentration of analyte in a sample was derived from a calibration graph obtained from the standards.

RESULTS AND DISCUSSION

Although the carbon furnace used was a standard commercial design produced to give highly sensitive atomic absorption measurements, it proved suitable for the atomic emission analysis of steels. Calibration graphs exhibit considerable curvature [1, 11] at concentrations 1.5—2 orders of magnitude above the detection limit. To achieve the best results with this apparatus, it was preferable to use different dilutions of the sample solution to achieve working concentrations (Table 1) which utilized the most linear portion of the calibration graph. Although subsequent work in the authors' laboratory has shown that maximum atomic emission signals are not always achieved at the maximum applied atomization voltage on the HGA 72, the operation of the instrument at the maximum setting of 999 digits gave a temperature of 2880 K (measured by optical pyrometer) with the modified tubes [3], which condition gave sensitive atomic emission signals suitable for the determination of the five elements investigated.

With detection limits in solution [3] of 2.6 ng Cr ml^{-1}, 2.1 ng Cu ml^{-1}, 4.4 ng Mn ml^{-1}, 1 ng Al ml^{-1} and 23 ng Ni ml^{-1}, it was clear that at least a ten-fold dilution of a 1% steel solution would be necessary in order to obtain solution concentrations within the most sensitive range of the method for the analysis of minor elements in steel (see Table 1). The interference of 1000 μg Fe ml^{-1} was therefore investigated on the determination of typical concentrations of the analyte elements. Since the samples were dissolved in nitric acid and finally made up in 0.8% nitric acid, the effect of the acid was also measured. The results are shown in Table 2. In each case, the appropriate tube background signal has been subtracted. Negligible interference of nitric acid and iron was observed for chromium, copper and manganese. Nitric acid produced a small depression of the nickel and aluminium signals but no further depression was observed on the addition of 1000 μg Fe ml^{-1}. Since there were small depressive effects from the combined effects of iron and nitric acid, standards were prepared containing the same concentrations of both as are present in the sample solutions.

TABLE 2

Effect of nitric acid and iron on the analyte emission signals

Analyte	Conc. in 50-μl aliquot (μg ml^{-1})	Signal, chart divisions		
		Aqueous soln.	0.8% HNO_3	1000 μg Fe ml^{-1} in 0.8% HNO_3[a]
Cr	0.2	42	40	40
Cu	0.2	61	62	60
Mn	0.2	39	38	39
Ni	0.5	30	26	25
Al	0.2	41	32	33

[a]Blank from 1000 μg Fe ml^{-1} and 0.8% HNO_3 at analyte wavelengths was zero in all instances.

TABLE 3

Determination of Cr, Cu, Mn, Ni and soluble Al in standard steel samples

Sample No.		Element				
		Cr (%)	Cu (%)	Mn (%)	Ni (%)	Al (%)
BCS 321	Results	0.110	0.021	0.120	0.102	0.110
		0.109	0.023	0.135	0.103	0.120
		0.108	0.024	0.110	0.100	0.108
		0.108	0.023	0.110	0.103	0.100
	Certificate	0.106	0.025[a]	0.13	0.099	0.100[a]
BCS 322	Results	0.042	0.021	0.270	0.199	0.080
		0.039	0.019	0.270	0.190	0.084
		0.038	0.018	0.280	0.190	0.086
		0.039	0.019	0.270	0.189	0.082
	Certificate	0.039	0.018[a]	0.28	0.194	0.088
BCS 325	Results	0.223	0.018	0.400	0.238	0.019
		0.230	0.016	0.401	0.219	0.018
		0.210	0.016	0.420	0.240	0.018
		0.209	0.019	0.400	0.236	0.020
	Certificate	0.220	0.018[a]	0.42	0.23	0.021
BCS 379	Results	0.026	0.050	0.271	0.040	
		0.025	0.053	0.268	0.041	<0.00012
		0.028	0.054	0.275	0.043	
		0.025	0.050	0.270	0.047	
	Certificate	0.028[a]	0.055	0.29	0.045	<0.00009[b]
BCS 330	Results	0.024	0.045	0.430	0.0082	0.014
		0.025	0.047	0.420	0.0079	0.014
		0.021	0.047	0.440	0.0080	0.011
		0.021	0.045	0.445	0.0083	0.013
	Certificate	0.020[a]	0.047	0.45	0.0086[b]	0.011

[a,b] When certificate values were not available, comparative values were obtained by flame atomic absorption spectrometry (a), or carbon-furnace atomic absorption spectrometry (b).

For all analytes, no signal was observed at the analyte wavelength for a blank solution containing 1000 μg Fe ml^{-1} and 0.8% nitric acid. Thus there is no spectral interference by iron on chromium (from the 427.18 and 421.62-nm iron lines), aluminium (from the 393.03-nm iron line) or any of the other elements. An ashing step designed to remove the nitric acid before atomization also appeared to be unnecessary. Samples were therefore simply dried and atomized.

Following these preliminary investigations, the procedures described in the experimental section were designed and tested on a number of standard steel samples. The results (Table 3) show excellent agreement between the carbon-furnace atomic emission method and the certificate values. Each result in Table 3 is the mean result from three separate injections of the same sample solution; the four results for each sample were obtained from

TABLE 4

Instrumental reproducibility for the determination of Cr, Cu, Mn, Ni and soluble Al in steel

	Cr	Cu	Mn	Ni	Soluble Al
Sample used, BCS	330	325	321	379	330
Certificate value (%)	0.020	0.018	0.13	0.045	0.011
Mean of 12 results (%)	0.020	0.017	0.130	0.043	0.012
S.d. (%)	0.0017	0.0008	0.0023	0.0020	0.0011
R.s.d. (%)	8.5	4.7	1.8	4.7	9.2

separate sample dissolutions. The reproducibility of the carbon furnace measurement was tested by repeated injections of a single sample solution for each element. The results (Table 4) give relative standard deviations between 1.8 and 9.2%. At the concentration levels tested, these would probably be more than adequate for analytical purposes or for the control of steel production or fabrication. With the exception of manganese, the relative standard deviations are slightly higher than would be expected for atomic absorption by either flame or carbon-furnace atomization. This may be a function of the dependence of emission signals on the temperature of the vapour phase in the furnace.

The results presented here demonstrate that accurate values can be obtained for the determination of five minor elements in steel by carbon-furnace atomic emission spectrometry. Only small chemical interferences are introduced by the sample solution matrix, which can be compensated by incorporation of this matrix in the standard solutions. There seems no reason to doubt that this technique could be applied to a number of other constituents of steels for which adequate sensitivity is available [3]. No spectral interferences were observed and, although manual subtraction of tube background was used here, automatic background subtraction could be introduced with appropriate instrumentation [2]. The reproducibility of the method could probably be improved with automatic sample injection as similar improvements have been obtained in carbon-furnace atomic absorption analysis [14].

Simultaneous multi-element analysis would obviously be possible with several types of spectrometer. The same set of atomization conditions was found to be optimum for the determination of all five elements but to achieve best sensitivity different elements had to be analysed at different sample dilutions. With present furnace designs, the use of a single sample solution would necessitate losses of sensitivity in some cases. In some analytical situations this might well be acceptable. However, improved linearity of calibration graphs might be achieved with different furnace designs or by using different or less sensitive wavelengths. These possibilities remain to be investigated.

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DYNAMIC RESPONSE STUDIES OF SOLID-STATE CHLORIDE-SELECTIVE ELECTRODES IN THE PRESENCE OF IRON(III) WITH AN ON-LINE COMPUTER

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SUMMARY

Under certain conditions, the presence of iron(III) in chloride solutions can cause erroneously high e.m.f. readings with solid-state chloride-selective electrodes. This anomalous behavior has been examined by monitoring the dynamic response of the iron(III)-perturbed electrode with an on-line computer. The magnitude of the analytical errors at equilibrium conditions depends on the iron(III) concentration, the chloride concentration, the extent of masking conditions and apparently the formulation of the sensing membrane. For such samples, markedly better analytical results can be obtained by extrapolating a segment of the dynamic response curve. The relative errors are reduced to 3—4% for samples where the linear extrapolation algorithm is valid. It is also possible to eliminate the interference by reduction to iron(II).

The desirability of making rapid measurements with solid-state ion-selective electrodes has led to a number of recent theoretical and experimental studies of their dynamic response characteristics, ranging from the early work by Pungor and co-workers [1, 2] to more recent studies [3—6]. In most cases, the rapid response profiles have been recorded as oscilloscopic traces of the analog signal. Several important advantages can be realized by monitoring the dynamic response with an on-line computer: a typical analog-to-digital converter possesses a wide dynamic range without sacrificing important resolution, the frequency of data acquisition can be altered during the experiment as the slope of the response curve changes, ensemble averaging can be used for noise discrimination [7], and the high-resolution data can be stored, output or massaged as required. The value of on-line computer data collection is particularly significant when numerous precise data points must be taken during the first few seconds of the response curve.

In addition to their relatively rapid response, an important characteristic of ion-selective electrodes is their selectivity. In the case of solid-state

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electrodes, an important class of interference involves the formation with a membrane component of a complex or precipitate which is more stable than that formed by the analyte. For example, sulfide, bromide, iodide, cyanide and ammonia can interfere with the silver chloride—silver sulfide solid-state chloride electrode [8]. A slightly different type of interference has been noted when silver sulfide-based electrodes are exposed to solutions containing both copper(II) and chloride ions: silver chloride and copper(II) sulfide are formed on the membrane surface [9]. The effects of chloride on the copper electrode [10] and of copper(II) on the chloride electrode [11] have been reported. Light and Swartz have described the successful use of a silver-sulfide membrane electrode as the end-point sensor for the titration of chloride in the presence of 0.1 M iron(III) [12].

The effect of iron(III)-containing solutions on the response of the silver chloride—silver sulfide solid-state chloride electrode is described in this paper. Although it is reportedly possible to use this electrode in the presence of iron(III) [8], anomalously high e.m.f. values were observed here under conditions where the iron(III) is not strongly masked. In citrate medium, the magnitude of this perturbation depends markedly on pH. This is illustrated by comparing the behavior of a solid-state chloride electrode (Orion EX-1 generation) in the presence and absence of 7×10^{-2} M iron(III) in solutions which contain 5.00×10^{-3} M chloride, 1.0 M sodium nitrate and 0.10 M citric acid. When the pH exceeds about 2.6, the presence of iron(III) does not affect the e.m.f. However, at pH values of 2.0 and 1.4, the e.m.f. readings are in error by 2 mV and 20 mV, respectively. This perturbation possibly results from an oxidative attack of the membrane. The dynamic response of the electrode during perturbation by iron(III) has been monitored here with an on-line computer.

The shape of the dynamic response curves can be rationalized in terms of the various time-dependent parameters contributing to the signal. The hypothetical example shown in Fig. 1B represents the rapid addition of iron(III) chloride solution to a chloride solution containing an equilibrated chloride electrode. The dynamic response of the electrode under these conditions can be viewed as a linear combination of normal response and perturbation by iron(III). The normal electrode response, represented by the lower segment of Fig. 1A, includes such factors as sample introduction rate, mixing rate and the inherent dynamic response time of the electrode. Rangarajan and Rechnitz [3] have shown the last factor to be about 0.2 s, extrapolated to a zero concentration step and infinite mixing rate. ΔE in Fig. 1A represents the equilibrium e.m.f. change caused by a decadic chloride concentration change in the absence of iron(III). The upper curve of Fig. 1A represents the type of response seen when the electrode is exposed to iron(III) without changing the chloride concentration. The early points appear linear; this is followed by curvature with decreasing slope until a steady state is reached after several minutes. A higher iron(III) concentration results in a larger e.m.f. change and also a more rapid loss of initial response linearity.

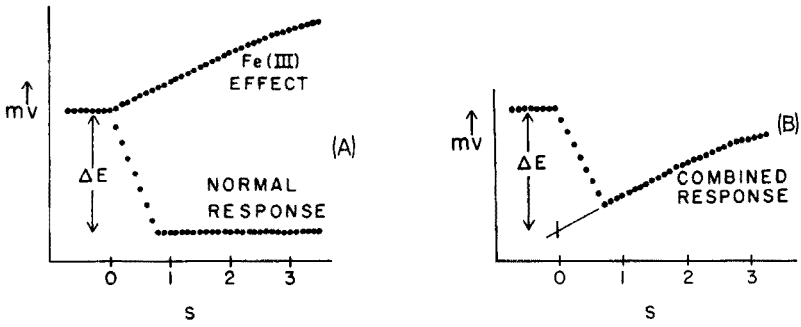

Fig. 1. Model of the dynamic response of solid-state chloride electrode in the presence of iron(III).

An empirical method has been developed for measuring chloride concentration in the presence of this electrode perturbation by iron(III). The technique involves the linear extrapolation of the initial positive slope of the combined response curve after the minimum to zero time to obtain the corrected e.m.f. change, ΔE. This approach (see Fig. 1B) is valid when the initial linear slope of the iron(III) effect persists beyond the minimum of the net curve, and the normal response factors are at steady state beyond the minimum. The on-line computer is important to this approach, both for high-quality data acquisition and rapid data manipulation and extrapolation.

EXPERIMENTAL

Reagents

The chloride solutions were prepared from primary standard-grade sodium chloride. All other chemicals were reagent grade. Deionized distilled water was used throughout. All solutions were made 1.0 M in sodium perchlorate or sodium nitrate to fix the ionic strength, and 0.10 M in citric acid to mask the iron with respect to the formation of chloride complexes. The pH adjustments were made with perchloric acid. The citrate concentration is more than a 10-fold excess over the value calculated to provide quantitative masking for the experimental conditions employed [13]. The chloride impurity in the electrolyte solutions was measured by Gran plots. The use of sodium nitrate electrolyte solution is preferred, since its chloride level of approximately 2×10^{-5} M is several times lower than for a corresponding sodium perchlorate solution.

Apparatus

The water-jacketed cell had the interior dimensions of a 100-ml beaker and was thermostated at 25.0 ± 0.1°C. The sample was injected into the cell with a 10-ml capacity Cole-Parmer precision digital dispenser; the injection times for 5.00- and 10.00-ml portions were 0.7 ± 0.1 and 1.1 ± 0.2 s,

respectively. These times were measured by injecting supporting electrolyte solution into chloride solutions containing an equilibrated chloride electrode. These injection times also include mixing time and inherent dynamic response factors. The cell contents were stirred with a glass propeller driven by a 600-rpm synchronous motor, which contributes much less noise than a magnetic stirrer to the electrode signal. Orion 94-17 solid-state chloride electrodes and 90-02-00 double junction reference electrodes were used. The computer was a Hewlett-Packard 2115B with 8K of core memory and equipped with a digital storage oscilloscope. The electrometer and interface have been described previously [7]. Manual measurements were made with a Corning digital electrometer. Controlled potential coulometry was done with a Princeton Applied Research Model 173 potentiostat.

Programming and procedure

All programming was done in a form of BASIC which includes assembly language subroutines for controlling timing, ADC operation, data acquisition and digital oscilloscope operation, and for monitoring the state of the switch register [7]. Each steady-state data point is actually the average of 167 ADC conversions taken at 10^4 Hz, which discriminates against 60-Hz line-noise pick-up [7]. Steady state was arbitrarily defined as no more than 0.1 mV e.m.f. change in 30 s, represented by three consecutive points taken at 10-s intervals. This criterion was used to select the calibration and equilibrium data to be retained in memory until the experiment is concluded.

The procedure consisted of three steps: electrode calibration, sample data acquisition with interactive linear curve-fitting, and linear least-squares treatment of calibration data followed by the calculation of the results. The experimental procedure is illustrated in Fig. 2. Before each experiment,

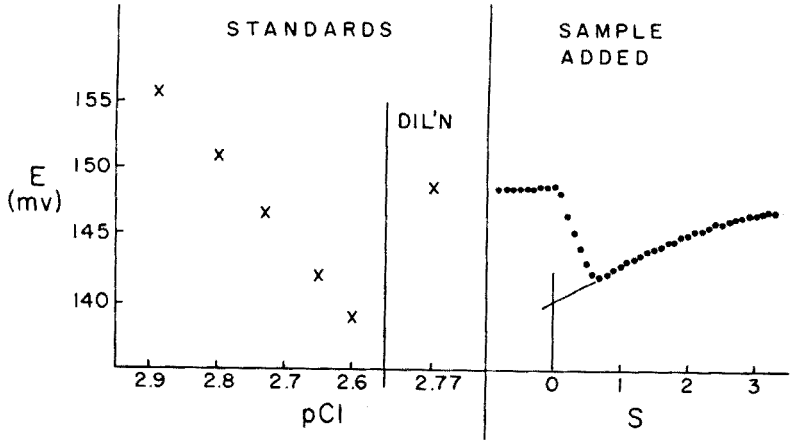

Fig. 2. Description of experimental procedure with on-line computer. Sample injected: 5.00 ml solution containing 5.00×10^{-3} M chloride, 3.5×10^{-2} M iron(III), 0.1 M citrate, 1.0 M sodium nitrate. pH 1.6.

information regarding data sampling frequency and standard concentration is entered via the Teletype. Electrolyte solution (10 ml) is pipetted into the cell and several additions of 0.1000 M chloride solution are made to calibrate the electrode. The last standard chloride solution is prepared by adding 5.00 ml of electrolyte solution to the cell, which brings the chloride concentration back to about two-thirds that of the highest standard solution.

After the calibration is completed, the computer requests Teletype entry of the initial data frequency desired for the sample (usually 10 Hz), the volume of sample to be injected (5.00 or 10.00 ml), and the theoretical iron(III) and chloride concentrations in the sample, if known. Data collection at the initial frequency is initiated by switch register command, which is followed immediately by rapid injection of the sample into the final standard solution. Sixty data points taken at the initial frequency are stored in memory, after which data are taken and printed every 10 s until a steady state is reached.

The 60 data points taken at the initial frequency are then tabulated and simultaneously plotted on the digital storage oscilloscope. After inspecting the plot and tabulation, the operator identifies and inputs the boundaries of the segment of the response curve to be linearized and extrapolated. Zero time is defined as the last data point before the potential excursion begins. The calculations are made via a linear least-squares subroutine, the output of which includes extrapolated zero-time intercept and its standard deviation, slope and its standard deviation, least-square residuals, and overall standard deviation. If any of the standard deviations or residuals are suspect, the linear segment can be re-defined and the extrapolation repeated as often as desired. The remainder of the program automatically computes the calibration parameters of the electrode and analytical results for both the extrapolated and steady-state data, including relative errors if known values for the sample had been entered. A copy of the program (CLISE IV-C) is available from the authors on request.

RESULTS AND DISCUSSION

Figure 3 illustrates the effect of iron(III) on the accuracy in terms of the relative errors seen for measurements made at steady state at pH 1.6. Each value was obtained by first calibrating the electrode in the absence of iron(III) and then allowing it to reach steady state in a chloride solution which also contained iron(III). The magnitude of the error is strongly dependent on the iron(III) concentration and, to a lesser extent, on the chloride concentration. A relative concentration error of —30% corresponds to an electrode e.m.f. error of about +10 mV. With the EX1 electrode, the onset of determinate errors was observed when the iron(III) concentration exceeded $0.1—0.2 \times 10^{-3}$ M. An older electrode (CX1 generation) gave errors only about 0.1 as large as those seen with the newer EX1 type, which suggests that the magnitude of the effect also depends upon the exact

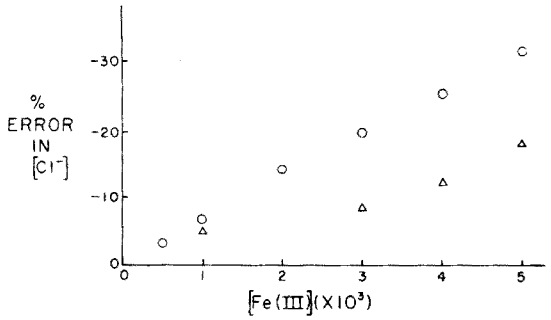

Fig. 3. Analytical errors in the presence of iron(III) under steady-state conditions. (○) 10^{-2} M chloride, (△) 10^{-4} M chloride.

formulation of the sensing membrane. Normal electrode response was restored by soaking the electrode in stirred, iron(III)-free 0.1 M chloride solution. The time required for restoration depended on the magnitude of the prior perturbation, but did not exceed 20 min for any of the experiments reported here.

A summary of the results obtained by on-line monitoring of the dynamic response is given in Table 1. Except in the absence of iron(III), the results obtained by computerized extrapolation of the response data are markedly better than those obtained at steady state. The errors appear to be somewhat larger for 5.0-ml samples than for 10.0-ml samples, but this is probably a result of attributing the total absolute error to a smaller fraction of the cell

TABLE 1

Results from on-line computer experiments

[Fe(III)], in sample (M)	[Cl^-], in sample (M)	Sample vol. (ml)[b]	%Relative error in sample [Cl^-][a]	
			By extrapolation	Steady state
0	5.00×10^{-3}	5	±3.4	−1.7
0	5.00×10^{-3}	10	±3.0	−0.8
5.0×10^{-3}	5.00×10^{-3}	5	±4.1	−13
5.0×10^{-3}	5.00×10^{-3}	10	±3.0	−22
1.5×10^{-2}	5.00×10^{-3}	5	±4.4	−47
1.5×10^{-2}	5.00×10^{-3}	10	±3.0	−60
2.5×10^{-2}	5.00×10^{-3}	5	±3.9	−45
2.5×10^{-2}	5.00×10^{-3}	10	±2.5	−44
3.5×10^{-2}	5.00×10^{-3}	5	±8.1	−93
3.5×10^{-2}	5.00×10^{-3}	10	±8.9	−95
5.0×10^{-3}	5.00×10^{-4}	10	−4.4	−11
1.5×10^{-2}	5.00×10^{-4}	10	−4.0	−43
3.5×10^{-2}	5.00×10^{-4}	10	−11.8	−89

[a]Results are the average of three or more trials at pH 1.6 with EX1 electrode.
[b]Sample is injected into 15 ml of final standard.

contents. The relative errors from the extrapolation technique at the lower chloride concentration (5×10^{-4} M) were always negative. Analogous experiments at this chloride level, but with no iron(III) in the sample, showed that the initial rapid response excursion (normal response segment of Fig. 1A) reaches an initial level about 0.5 mV more positive than the steady-state value, which is reached only after about 1 min. Therefore, a distinct determinate error appears at low concentrations where inherent response is slow.

The errors in the extrapolated results are largest at the highest iron(III) concentration where linearization was least certain. Since this observation is mirrored in the steady-state results, it could be related to the markedly larger perturbation effect seen at this iron(III) concentration. This was studied by examining the electrode response to the addition of 3.5×10^{-2} M iron(III) without producing a chloride gradient. The ideal response would be similar to the top curve of Fig. 1A, but the actual response was non-linear throughout, accounting for the large experimental uncertainty at this iron(III) concentration.

In summary, this corrective technique yields very acceptable results for strongly acidic samples under conditions where the dynamic response model is valid. For samples whose pH can safely be raised, masking by citrate will eliminate the perturbation by iron(III). Reduction of the iron(III) to iron(II) also is useful for eliminating the interference if the sample must remain strongly acidic. A series of pH 1.0 iron(III)—chloride mixtures containing no masking agent were reduced by controlled-potential electrolysis under oxygen-free conditions. The e.m.f. values for samples containing as much as 0.04 M iron agreed within experimental precision with those observed for the corresponding iron-free chloride solutions, showing neither electrode perturbation nor interference by formation of chloro complexes of iron(II) [11].

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RAPID DETERMINATION OF NICOTINIC ACID BY IMMOBILIZED *LACTOBACILLUS ARABINOSUS*

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SUMMARY

A rapid determination of nicotinic acid by using immobilized *Lactobacillus arabinosus* and a combined glass electrode is reported. *L. arabinosus* is immobilized in agar gel. The optimum agar concentration is from 2—2.5% (w/v) and the optimum bacterial concentration in the agar gel matrix is 10 mg wet cells ml^{-1}. The relationship between the potential difference and the logarithm of the nicotinic acid concentration is linear over the range 5×10^{-8}—5×10^{-6} g ml^{-1}. The assay requires only 1 h; potentials are reproducible with an average relative error of 5%. The growth of *L. arabinosus* in agar gel matrix is observed in the medium containing nicotinic acid. Immobilized *L. arabinosus* is stable for 30 days.

Vitamins and antibiotics are usually determined by microbioassay [1], based on turbidimetric or titrimetric methods. However, these methods require a long time for cultivation of bacteria. A faster microbioassay of vitamin B_1 by using a new electrode which consisted of a platinum anode and silver peroxide, has been reported recently [2]; electrochemical microbioassay of vitamin B_1 is thus possible within 6 h.

In recent years, many analytical methods involving enzymatic reactions have been developed by using immobilized enzyme and electrochemical monitoring [3, 4]. Techniques for immobilizing intact microorganisms have been reported [5, 6] and these immobilized microorganisms have been applied in microbial electrodes for the estimation of biochemical oxygen demand (BOD) [7, 8]. These immobilized microorganisms are active in a polymer matrix for one month. In this case, non-specific utilization of nutrients is required for the immobilized microorganisms. In contrast, some bacteria require specific nutrients for growth; these bacteria produce mainly lactic acid as a metabolite. A rapid method for determination of nicotinic acid, by using immobilized bacteria and a combined glass electrode to measure the lactic acid produced, is described here.

EXPERIMENTAL

Materials

The basic assay medium (Takara Kosan Co.) has the composition shown in Table 1. Nicotinic acid (Wako Pure Chemicals), agar (Kyokuto Pharmaceutical Co.), acrylamide and *N,N'*-methylenebisacrylamide (Wako Pure Chemicals) were used as received. Collagen was obtained from steer hide; the skin collagen was purified as described previously [9]. Other reagents were commercially available analytical reagents or laboratory-grade materials. Deionized water was used in all procedures.

Culture of microorganisms

Lactobacillus arabinosus ATCC 8014 was employed for the assay of nicotinic acid. It was maintained in peptone-yeast agar and transferred to a fresh medium every 10—14 days. The bacteria were cultured under anaerobic conditions at 37°C for 16 h in 500 ml of medium (pH 6.80) containing 2.5 g of yeast extract, 5.0 g of peptone, 5.0 g of glucose, 125 mg K_2HPO_4, 125 mg KH_2PO_4, 50 mg $MgSO_4 \cdot 7H_2O$, 2.5 mg $FeSO_4 \cdot 7H_2O$, 2.5 mg $MnSO_4 \cdot H_2O$, and 2.5 mg NaCl. The cells were centrifuged at 5°C and 8000 G, and washed twice with physiological saline.

Immobilization of microorganisms

For the entrapment of bacteria in the agar gel, 20 mg of agar was dissolved in 900 μl of physiological saline in a test tube at 100°C, and cooled to 50°C. Then, physiological saline (100 μl) containing 10 mg of wet intact cells of *L. arabinosus* was added, and the mixture was cooled to 37°C. To this immobilized bacteria gel, 1 ml of medium (pH 6.80) was added; the test tube was then kept at 30°C for 12 h. Finally, the immobilized bacteria were stored at 5°C.

TABLE 1

Composition of the assay medium
(Double strength, 100 ml, pH 6.8)

Casamino acid	1 g	Adenine	1 mg
L-Cysteine	40 mg	Guanine	1 mg
L-Tryptophan	10 mg	Uracil	1 mg
Glucose	2 g	Xanthine	1 mg
CH_3COOK	2 g	Thiamine	0.1 mg
K_2HPO_4	50 mg	Riboflavin	0.1 mg
KH_2PO_4	50 mg	Pyridoxin	0.1 mg
$MgSO_4 \cdot 7H_2O$	20 mg	Pyridoxal	0.1 mg
$FeSO_4 \cdot 7H_2O$	1 mg	Pantothenate	0.1 mg
$MnSO_4 \cdot 4H_2O$	1 mg	*p*-Aminobenzoic acid	0.02 mg
NaCl	1 mg	Biotin	0.001 mg
NH_4Cl	300 mg	Folic acid	0.001 mg

For the entrapment of bacteria in the polyacrylamide gel, 45 mg of acrylamide and 5 mg of *N,N'*-methylenebisacrylamide were added to 1 ml of physiological saline containing 10 mg of wet intact cells, and the system was blanketed with nitrogen. Polymerization was then initiated with 20 μl of dimethylaminopropionitrile and 1.25 mg of potassium persulfate, and allowed to proceed for 30 min at 37°C. The immobilized bacteria were left in contact with 1 ml of medium for 12 h, and stored at 5°C.

Bacteria were entrapped in the collagen membrane by the casting method described previously [8]. A suspension containing 10 mg of collagen fibrils and 10 mg of wet cells was employed. The bacteria—collagen membrane was treated with 1% glutaraldehyde solution (pH 7.0) for 1 min at 20°C.

Assay procedure

Aliquots (1 ml) of the double-strength basic assay medium and 1-ml aliquots of sample solutions containing appropriate amounts of nicotinic acid were placed in test tubes containing 1 ml of immobilized bacteria gel prepared as described above. As a blank test, 2 ml of the assay medium was placed in another test tube with 1 ml of the bacteria gel. For incubation, all these test tubes were thermostated at 30°C for 1 h. The potentials were then measured by inserting a combined glass electrode (TOA electrode, GST-155C) in each test tube; a TOA MH-5BS pH meter was used to measure potentials and the signals were displayed on a recorder (Riken Denshi, Model SP-J3C). The potential differences between the medium containing nicotinic acid and the blank medium were calculated.

Determination of bacterial growth in the gel

The agar gel containing *L. arabinosus* was crushed and ground in a mortar. Viable cell counts were made by spreading appropriate dilutions and counting colonies after incubation for 24 h at 30°C.

RESULTS

Preliminary experiments showed that the immobilized *L. arabinosus* produced mainly lactic acid. As the membrane potential of the glass electrode is proportional to the logarithm of proton activity in the solution, the lactic acid produced by the immobilized whole cells can be determined by the electrode. The potential increased with increasing concentration of nicotinic acid in the medium. The potential difference (ΔE_1) between the initial medium and the medium incubated for 1 h with immobilized bacteria was calculated. The potential difference (ΔE_2) between the incubated blank and sample media was also calculated. The rate of production of lactic acid by immobilized bacteria in the media containing nicotinic acid was higher than that in the blank medium.

The potential difference (ΔE) between ΔE_1 and ΔE_2 was found to be proportional to the logarithm of the nicotinic acid concentration:

$$\Delta E = \Delta E_1 - \Delta E_2 = k \log [\text{nic}]$$

where ΔE is a potential difference, [nic] is the nicotinic acid concentration and k is a constant.

Screening of carrier for immobilization

L. arabinosus was immobilized in agar gel, polyacrylamide gel and collagen membrane. Table 2 shows the potential differences (ΔE) obtained from the bacteria immobilized in various carriers. The bacteria immobilized in the agar gel gave the largest potential difference, and this method was used for all subsequent work. The potential differences obtained from the bacteria immobilized in the polyacrylamide gel and the collagen membrane were very small.

Optimum conditions for immobilization

Figure 1 shows the effect of agar concentration on the potential difference. The maximum potential difference was observed at agar concentrations between 2% and 2.5% (w/v); outside that range, changes in the agar gel concentration decreased the potential difference.

The effect of bacteria concentration in the agar gel on the potential difference is shown in Fig. 2. The optimum bacteria concentration was 10 mg wet cells ml^{-1}. The potential difference became very low at high concentrations of bacteria.

Time course of the reaction

The time course of the reaction is shown in Fig. 3. The potential difference first increased linearly with increasing incubation time, but reached a plateau after incubation for 1 h, because both ΔE_1 and ΔE_2 then changed at the same rate. An incubation time of 1 h was selected for the assay of nicotinic acid.

Calibration curve

The relationship between the potential difference and the logarithm of the nicotinic acid concentration was examined. A linear relationship was obtained for 5×10^{-8}—5×10^{-6} g of nicotinic acid in the 1-ml aliquot added

TABLE 2

Potential differences (in mV) obtained after immobilization of bacteria in various gel matrices

	ΔE_1	ΔE_2	ΔE ($\Delta E_2 - \Delta E_1$)
Native	67.3	70.0	2.7
Collagen membrane	52.0	52.2	0.2
Polyacrylamide (acrylamide 9%, BIS 1%)	85.0	93.2	8.2
Agar	70.2	99.7	29.5

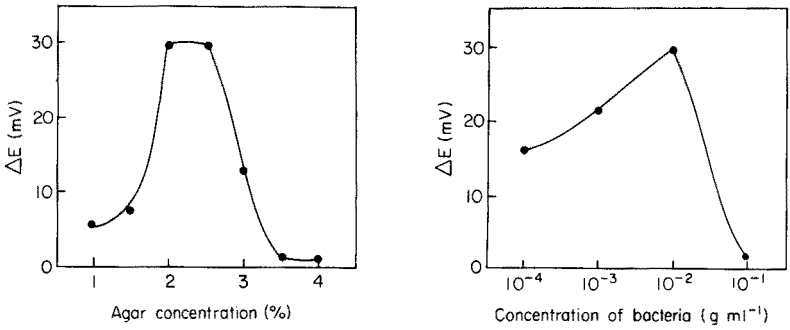

Fig. 1. Effect of agar concentration on the potential difference. The medium contained 5×10^{-6} g of nicotinic acid per ml. The bacterial concentration was 10^{-2} g of wet cells per ml. The potentials were measured after incubation for 1 h at 30°C.

Fig. 2. Effect of bacteria concentration on the potential difference. The agar concentration was 2% (w/v) and the other experimental conditions were the same as for Fig. 1.

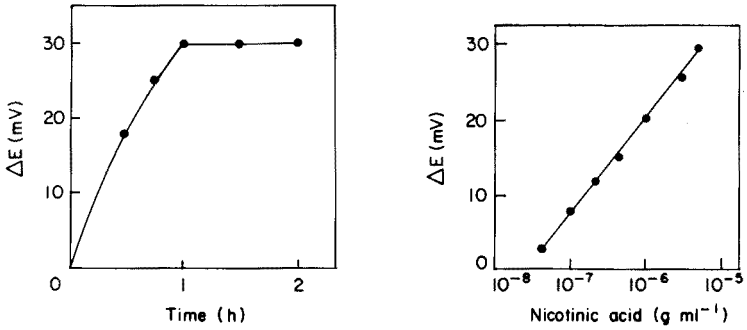

Fig. 3. Time course of potential difference. The bacterial concentration was 10^{-2} g of wet cells per ml. The other experimental conditions were the same as for Fig. 2 except for incubation time.

Fig. 4. Calibration curve for nicotinic acid under optimum conditions.

(Fig. 4). The potential difference was reproducible with an average relative deviation of 5% when a medium containing 5×10^{-7} g of nicotinic acid per ml was employed; the standard deviation was 2×10^{-8} g ml^{-1} in 30 experiments.

Storage stability of the immobilized bacteria

To study the stability of the immobilized bacteria, they were stored in the physiological saline at 5°C. Nicotinic acid was determined at 10-day intervals with this stored immobilized bacteria. The potential difference obtained from each experiment was constant for 30 days. The bacteria immobilized in agar gel matrix are therefore active for a month.

Determination of bacteria growth in agar gel

Growth of the bacteria in the agar gel was examined by the method described under Experimental. The number of bacteria in the gel increased from 4.1×10^{10} to 5.6×10^{10} g^{-1} of gel during incubation in the medium containing nicotinic acid. Without nicotinic acid, growth of *L. arabinosus* in the gel was not observed.

Chloramphenicol is known as an inhibitor of protein synthesis in bacteria; its effect was therefore examined. The potential difference obtained from a standard medium containing 5×10^{-6} g of nicotinic acid per ml was 29.5 mV, whereas the potential difference decreased to 4.1 mV when a medium containing 10 μg of chloramphenicol per ml was employed for the assay. Chloramphenicol thus inhibits growth of the bacteria.

DISCUSSION

L. arabinosus requires nicotinic acid for its growth in the medium used. The microbioassay of nicotinic acid is based on this growth. As previously reported [2], a large injection of bacteria shortened the time required for the microbioassay. However, the minimum time was still 6 h when 5×10^{-6} g of wet cells per ml was injected into the incubation medium, and increased injections did not shorten the incubation time further. This suggests that the bacteria cannot grow in a medium containing a large amount of the bacteria. A rapid microbioassay is still desirable for practical use. It was found that the bacteria could grow in gel containing a large amount of bacteria. Furthermore, as previously reported [5—7], immobilized bacteria are active for a long time.

The potential difference measured was small when the bacteria were immobilized in polyacrylamide gel or collagen membrane. The total concentrations and relative ratio of acrylamide and *N,N'*-methylenebisacrylamide determine both the pore size of the interstitial space within which bacteria are entrapped and the physical nature of the gel. Immobilization of *L. arabinosus* was done under the optimum conditions reported previously [5]. Therefore, the small potential difference may be due to repression of the bacterial growth by the crosslinkage of the polyacrylamide gel. As the collagen membrane was also crosslinked with glutaraldehyde, growth of the bacteria in this membrane may be repressed for the same reason. In contrast, the potential difference increased when the bacteria were immobilized in agar gel. Agar is a natural material with ill-defined crosslinkage, and the bacteria can grow. This result was confirmed by the effect of addition of chloramphenicol to the cultivation medium. Growth of the bacteria depends on the pore size of the interstitial space. The optimal agar concentration for immobilization was 2% (w/v); further increase of the agar concentration decreases the pore size of the interstitial space, and the bacteria cannot grow under these conditions. However, the bacteria may leak from a gel of low concentration.

An optimal concentration of bacteria and a constant potential difference obtained after incubation for 1 h can be explained similarly. The interstitial space becomes filled with bacteria at a higher concentration of bacteria, so that further growth becomes impossible and smaller changes in potential difference are obtained. The bacterial population in the gel also reaches a maximum after incubation for 1 h, and a constant potential difference is obtained.

In conclusion, the determination of nicotinic acid is possible within 1 h by using immobilized bacteria. This method can be also applied to the determination of other vitamins, amino acids, and antibiotics.

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AUTOMATED IMMUNOASSAY WITH A SILVER SULFIDE ION-SELECTIVE ELECTRODE

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SUMMARY

Antibody levels in the $\mu g\ ml^{-1}$ range are selectively and rapidly determined in an automated flow system which employs an Ag_2S membrane electrode sensor in conjunction with immobilized immunoabsorbent. Experiments with anti-benzoate antibody in the presence of excess γ-globulin or whole serum demonstrate the operating characteristics of the system in terms of selectivity, precision and dynamic response.

Quantitative immunoassay has been in general use since Heidelberger and Kendall [1] introduced the precipitin test for the determination of antibodies and antigens. This was the first method adequately to fulfill the basic criteria of specificity, accuracy, reproducibility and sensitivity. More recently, single radial immunodiffusion, double immunodiffusion, immunoelectrophoresis, radioimmunoassay [2] and enzyme immunoassay [3, 4] have been used in the quantitative measurement of antibodies.

Nisonoff and co-workers [5, 6] introduced the use of immunoabsorbents for antibody quantification. The sample of interest was passed over the immunoabsorbent which was then washed to remove any non-specifically absorbed materials. The specifically absorbed antibody was eluted with an appropriate buffer solution and determined by an acceptable technique, e.g. the Lowry method [7]. Considerable progress has been reported with the use of cellulose [8], polyacrylamide [9—11], polysaccharides [12] and agarose [10] as supports for immunoabsorbents. Immunoabsorbents have also been coupled with enzymatic methods of detection [13].

It was recently shown [14, 15] that Ag_2S membrane electrodes can be used for the determination of proteins. The method is based on the electrode response to the sulfhydryl groups exposed during alkaline denaturation of the protein followed by treatment with silver ions. The potential change can be related to the quantity of protein present. The same basic technique was also used to monitor in a semi-automated mode the precipitin curves of antigen—antibody reactions [16].

This paper describes an automated system which employs an immuno-absorbent in conjunction with the Ag_2S membrane electrode to measure

antibody concentrations in flowing samples. The technique permits determinations to be made with sensitivity and reproducibility while providing ease of operation through automated sample handling and treatment.

EXPERIMENTAL

Apparatus

A Technicon Sampler II and proportioning pump were used as components of the automatic flow system. An Orion model 94-16A Ag_2S indicator electrode with flow cap was used to monitor silver ion concentrations in the flowing stream. An Orion model 90-02-00 double-junction electrode was used as reference, and the stream was grounded with a platinum wire. The potentiometric output was recorded on a Beckman model 1055 pH/mV recorder.

Reagents

The *p*-aminobenzoic acid, bovine serum albumin (Cohn Fraction V) and cyanogen bromide-activated Sepharose 4-B were from the Sigma Chemical Co. The affinity-purified anti-benzoate antibodies and non-reactive γ-globulins were kindly provided by the Roswell Park Memorial Institute (Buffalo, N.Y.). The Calibrate serum control (General Diagnostics) was prepared as per label instructions. Other chemicals were Fisher reagent grade.

Procedures

The immunoabsorbent was prepared by a modified procedure [12]. Cyanogen bromide-activated Sepharose 4-B (1 g) was mixed with 400 mg of bovine serum albumin (BSA) in 20 ml of a 0.1 M $NaHCO_3$—0.9% NaCl buffer at pH 9.0 and stirred for 72 h at 4°C. The Sepharose-BSA conjugate was washed with 0.9% (w/v) NaCl solution until the absorbance at 280 nm was $\leqslant 0.01$.

The *p*-aminobenzoic acid was diazotized with nitrous acid in an ice bath by standard methods. To neutralize any excess of nitrous acid, sufficient 1.0 M sulfamic acid was added and the pH was adjusted to 5.5 with 1.0 M NaOH solution. After centrifugation at 1000 G for 5 min, 1.8 g (wet wt.) of the Sepharose-BSA gel was added and stirred for 24 h at 4°C. The gel was packed into a 1.0-ml Tuberculin syringe to a level of 0.3 ml. Plastic adapters were cemented into the ends of the syringe barrel for incorporation into the flowing stream of the AutoAnalyzer. A micro-debubbler was designed to prevent entry of air bubbles into the column.

The anti-benzoate antibody was diluted to concentrations of 100—1000 $\mu g\ ml^{-1}$ in solutions either 0.9% NaCl, 0.9% NaCl with non-reactive γ-globulin added or Calibrate serum control diluted 1:10 or 1:20 with 0.9% NaCl.

The urea/silver nitrate stock solution was 5.0 M in urea and 6.0×10^{-5} M in silver nitrate. The sodium hydroxide stock solution was 2.0 M. The glycine—HCl and glycine—NaOH washes were made up to 0.1 M, with chloride concentration set at 0.9% with NaCl and adjusted to pH 2.8 and 10.0, respectively. These solutions were prepared with distilled-deionized water.

The flow system was operated in a manner similar to that previously described for total protein analysis [15], except that the immunoabsorbent column (with micro-debubbler) was inserted into the sample line between the sampler module and the proportioning pump. For antibody determinations, the sampler cam was also adjusted to provide a 2-min acidic glycine wash. The continuous flow auto-analysis manifold for this system is shown schematically in Fig. 1. The column was conditioned with the alkaline glycine wash and samples were then aspirated. The NaCl rinse and acidic glycine eluent were subsequently drawn into the system. Between sample cups on the Sampler II module, 0.9% NaCl solution was aspirated from the wash receptacle. (The cyclic analysis scheme is shown diagrammatically in Fig. 5.)

RESULTS

The calibration curves for anti-benzoate antibodies and non-reactive γ-globulins are shown in Fig. 2. These curves illustrate the changes in potential ($-\Delta E$) obtained for total protein analysis as previously described [15].

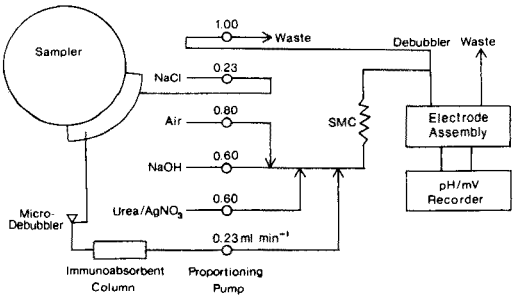


Fig. 1. Schematic diagram of the continuous flow apparatus. Concentrations used throughout were: Ag^+ (6.0×10^{-5} M), urea (5.0 M), NaCl (0.9%). The cam was a modified 1:1 sample-to-wash at 20 samples per h.

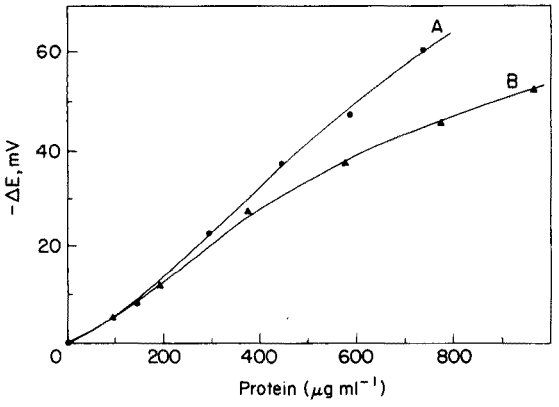


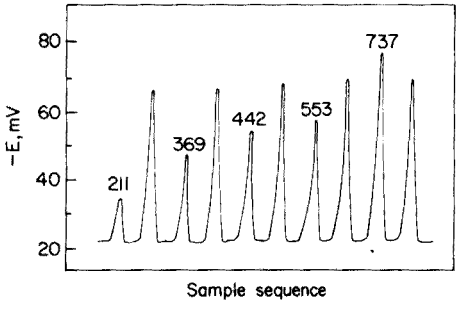
Fig. 2. Calibrations for total protein analysis. (A) Anti-benzoate antibodies, (B) γ-globulins.

The immunoabsorbent column was inserted for the analysis of anti-benzoate antibodies spiked with a fixed amount of non-reactive γ-globulins. The complete sequence of precondition wash, sample, NaCl rinse and acidic eluent was used. The γ-globulins were not retained by the column and appeared with a constant $-\Delta E$. The antibodies were resolved by the immunoabsorbent column so that their response appeared after that of the γ-globulins. As a result, the potentiometric output of the system consisted of alternating γ-globulin and antibody peaks (Fig. 3).

This procedure was repeated with reconstituted serum controls in place of the γ-globulins. It was decided to use low concentration levels of antibody in this series and then combine both sets of data to simulate varying background levels of non-specific proteins. In this manner, a selective response to the desired antibody can be achieved and, from the peak heights, a calibration curve for antibody response can be constructed. This curve is shown in Fig. 4 along with the calibration curve for anti-benzoate antibodies alone without a column. It can be seen that an excellent correlation exists for these artificial samples containing either fixed amounts of γ-globulins or unknown amounts of proteins with increasing amounts of anti-benzoate antibodies.

DISCUSSION

The sensitivity of the system covers a broad range of immunoglobulin concentrations. If however, a sample falls outside the concentration limits of the system, simple dilution of the sample or replacement of the sample pump tubing to increase or decrease sample size may be used to change appropriately the sensitivity of the system. Since the electrode sensor ultimately responds to the sulfhydryl content of the antibody involved, the slope of the



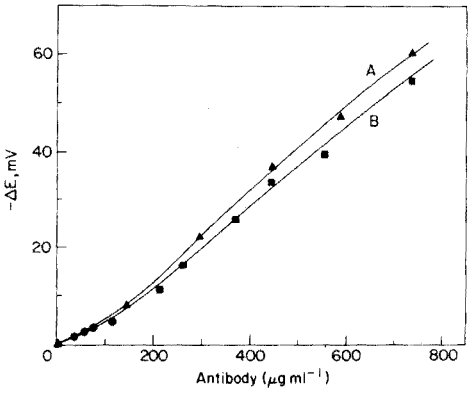


Fig. 3. Potentiometric recording for analysis of antibody mixed with γ-globulin. γ-Globulin concentration, 965 μg ml^{-1}; antibody concentrations given in μg ml^{-1}.

Fig. 4. Antibody calibrations. (A) No γ-globulins, no column used. (B) γ-Globulins (■) or serum controls (●) used with a 0.3-ml column.

calibration curve and the magnitude of the potential changes will depend on the chemical nature of the antibody molecule. For antibodies with high sulfhydryl content, the sensitivity of the method extends below the $\mu g\ ml^{-1}$ level. For example, anti-bovine serum albumin antibodies have been determined at levels approaching 100 ng ml^{-1} with the system described [17].

The precision of the method can be estimated directly from Fig. 4. The difference in $-\Delta E$ between the two curves shows a deviation of 6—15% with a mean of 11%. This deviation is fairly constant throughout the concentration range reported and, by using the appropriate calibration curve, specific antibodies can easily be determined with the necessary precision.

The scheme in Fig. 5 illustrates the cyclic nature of the automated immunoassay system. The immunoabsorbent is first ionized by an alkaline prewash to enhance the antibody—hapten interaction. The sample is introduced and the specific antibody is absorbed. Following a saline rinse, all other macromolecules, both unretained and physically retained, are washed out. An acidic glycine wash is used to elute the absorbed antibody, which is determined in the flow analyzer. The absorbent is thus "regenerated" and is ready to receive a fresh sample. This is a useful quality in repetitive measurements since costly immunogens are not consumed. Furthermore, the automated cyclic procedure yields an antibody determination every 12 min.

The proposed method thus offers speed, low cost, selectivity, sensitivity and acceptable precision. It should also be noted that the general approach of the method could be readily extended to other immuno-agents by the use of appropriate columns and/or electrodes in the system.

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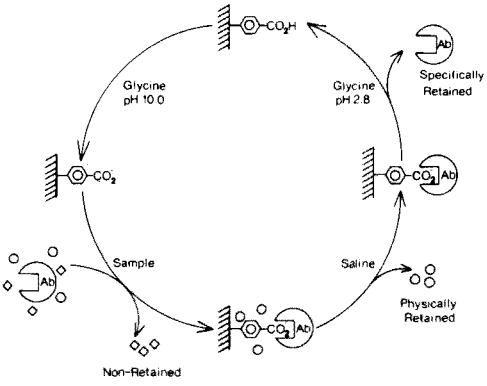

Fig. 5. Immunoabsorbent flow diagram. Conditions described in text; ○ and □ represent non-specific macromolecules in the sample; Ab, antibody.

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A COATED-PLATINUM SULFATE-SELECTIVE ELECTRODE

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SUMMARY

Sulfate-selective electrodes can be fabricated from commercial platinum electrodes by coating them with a mixture of Aliquat-336 and trifluoroacetyl-*p*-butylbenzene in polyvinyl chloride matrix. Adding a solid, sparingly soluble sulfate such as that of strontium, lead, barium, or 2-aminoperimidine (2-AP) improves the selectivity for sulfate relative to nitrate. The 2-AP electrode shows the best physical stability and is described in detail. The order of anion interference is $ClO_4^- >> Cl^- > NO_3^- > H_2PO_4^- > F^-$. The useful pH range is 4—9. The response is Nernstian from 10^{-5} to 10^{-1} M in sodium sulfate solutions.

Development of ion-selective electrodes for polyvalent anions like sulfate has been of continuing interest. An early contribution to this development was the barium sulfate-impregnated, silicone rubber-based electrode [1]; recently, another heterogeneous electrode of barium sulfate in polyvinyl chloride was described [2]. A different type is a crystal membrane electrode [3, 4] that consists of a mixture of metal sulfides and lead sulfate fused under pressure. A sulfate electrode was also prepared in a series of coated-wire electrodes [5], which consisted of an anion exchanger—polyvinyl chloride coating on platinum wire.

This paper describes a sulfate-selective electrode that is an extension of the coated-wire electrode. Fabrication is simplified by using a commercial platinum electrode as the unit of construction. Including a sparingly soluble sulfate in the coating mixture improves selectivity. Several inorganic sulfates were tried as additives, but 2-aminoperimidine sulfate [6] was the most satisfactory. The simplicity of fabrication may provide a pattern for laboratory preparation of electrodes for other ions.

EXPERIMENTAL

Reagents

Chemicals were reagent-grade, and the solutions were made with deionized water.

2-Aminoperimidine hydrochloride (2-AP chloride; $C_{11}H_9N_3 \cdot HCl$) was synthesized by PCR, Inc., as described by Stephen [6]. The product received

was recrystallized from methanol. Trifluoroacetyl-*p*-butylbenzene (TFAB; p-C_4H_9-$C_6H_4COCF_3$) was synthesized by PCR, Inc., as described by Herman and Rechnitz [7]. Aliquat-336 was converted to the sulfate form by four extractions with 1 M Na_2SO_4. A 50% (v/v) solution in TFAB was prepared, to which anhydrous sodium sulfate was added as desiccant.

Poly(vinylchloride) (PVC) and tetrahydrofuran (THF) were used as received from Aldrich Chemical Company, Inc.

Apparatus

Potential measurements were made with either an Orion Research Digital pH/mV Meter, Model 801A, or a Beckman SS-2 Expandomatic pH Meter. A Beckman platinum inlay electrode (No. 39273) was coated with the mixture described below. The reference electrode was Orion double-junction reference electrode (No. 900200) with 0.1 M KF in the outer chamber.

Preparation of electrodes

The 2-AP sulfate salt was precipitated by adding 2-AP chloride solution to excess of sodium sulfate. The sulfate of barium, strontium, or lead was similarly precipitated by adding the metal nitrate. Each was washed with water, oven-dried at 110°C, and pulverized in an agate mortar. The coating mixture was prepared from these sulfates by two procedures.

Procedure 1. For the initial experiments, the inorganic sulfates were sized by passing them through a U.S. standard sieve No. 200; the 2-AP sulfate could not be sieved because it caked. The coating mixture was prepared by grinding 0.075 g of sulfate salt and 5 drops of the Aliquat—TFAB solution in an agate mortar to form a paste. Then 15 drops of 10% (w/v) PVC in THF were added, and the mixture was ground together again.

Procedure 2. Later the coating mixture was prepared with a mixer/mill (Spex Industries, Inc.) by using an agate vial and a glass ball. First, 0.075 g of the sulfate salt was mixed for 6—10 min, 5 drops of Aliquat—TFAB were added, and the mixing was continued for about 6 min. Then 0.1 g of solid PVC was added and mixed for another 10 min. The solid mixture was transferred to a glass vial with a polyethylene-lined cap and was dissolved in 1—2 ml of THF using a vortex mixer.

With both of the above coating mixtures, more THF was added when necessary to obtain a consistency suitable for transfer. Then several drops (about 1/3 of the coating mixture) were placed on the platinum surface and over the edges of the inverted electrode.

The coating was allowed to dry overnight. The new electrodes were conditioned by soaking then in ca.10^{-2} M Na_2SO_4 until a stable reading was obtained (15—30 min). Then the edges of some electrodes were sealed with Parafilm. The electrodes were stored dry; they were reconditioned in Na_2SO_4 before use.

Measurement conditions

Measurements were made at room temperature (ca. 24°C) in 20 ml of magnetically stirred solution in a 50-ml beaker. Calibration curves were obtained by successive additions to the stirred solution of Na_2SO_4 (e.g., 0.1 M) from a microburet.

RESULTS AND DISCUSSION

Initially, coated-wire electrodes for sulfate were prepared by the procedure of Freiser and co-workers [5]. However, the platinum wire electrodes had to be specially constructed, and the coated electrodes were not uniformly satisfactory. Fabrication was then simplified and improved by coating the platinum surface of a commercial platinum-inlay electrode. The sulfate response was similar for the two types of electrodes.

Selectivity for sulfate relative to nitrate was poor, however, with both the coated-wire and coated-platinum electrodes. Presence of a sulfate salt, in addition to the ion exchanger, was expected to enhance the electrode response in favor of sulfate; therefore, some slightly soluble sulfate salts (lead, strontium, barium, and 2-AP) were tried as additives to the Aliquat—PVC mixture.

Barium and strontium sulfates appeared to be somewhat soluble in the coating mixture. The particles of 2-AP sulfate, however, formed a fairly stable suspension. The size of individual particles of 2-AP, determined by scanning electron microscopy, was generally less than 1 μm.

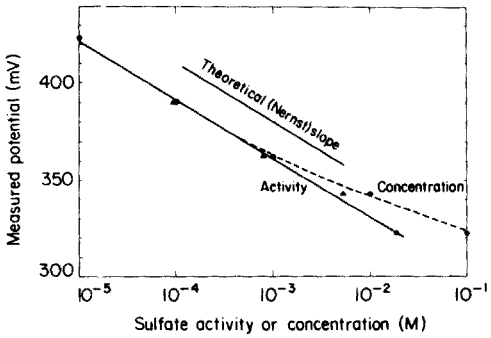
The requisite particle size and degree of dispersion of the solids were apparently attained with both procedures for grinding and mixing. Barium and 2-AP electrodes, prepared both ways, behaved in a similar manner.

The membranes with lead and strontium sulfates were less stable than those with barium and 2-AP. The barium sulfate membrane deteriorated more in aqueous solution than the 2-AP membrane. Therefore, only the 2-AP electrode was investigated in detail.

Another variant was the solvent for the Aliquat salt. When decanol was used [5], the coating did not adhere firmly to the platinum surface. TFAB, which was the solvent for the carbonate liquid membrane electrode [7, 8], produced a more adherent coating. Membranes that were made with TFAB without solid additions eventually turned yellow, but this did not affect their response to the sulfate ion.

Electrode characteristics

Response. The response of the 2-AP electrode to sodium sulfate solutions (Fig. 1) shows that the Nernst relationship is obeyed throughout the range 10^{-5}—10^{-1} M. Measured concentration was converted to ion activity as given by Kielland [9] for sodium sulfate. The linearity of the activity—potential plot confirms these calculated activity values, particularly at higher concentrations, and the response of the electrode to activity of the sulfate ion. Electrodes with and without added $BaSO_4$ gave calibration curves similar to Fig. 1.



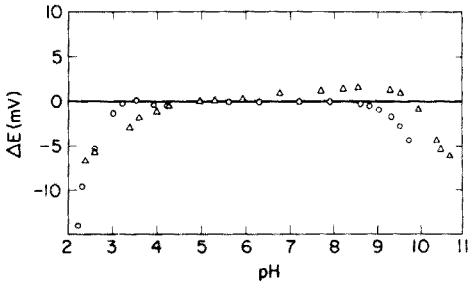


Fig. 1. Response of sulfate-selective electrode (2-AP) to Na_2SO_4.

Fig. 2. Effect of pH on sulfate-selective electrode (2-AP). (○) 5×10^{-3} M Na_2SO_4 + NaOH or HCl; (△) 10^{-2} M H_2SO_4 + NaOH.

Selectivity. Because of the non-ideal behavior of the electrode, meaningful selectivity coefficients could not be calculated. Instead, selectivity of the electrode relative to other ions was investigated through construction of calibration curves. The potential of the electode was measured in an electrolyte solution, when additions of sulfate were made. The background electrolyte affected the electrode response in the following ways: (1) a large negative shift in potential (usually reproducible within 5 mV for a given electrode) from that of the electrode in distilled water; (2) a region of non-linear response to sulfate at lower concentrations, followed by a linear response above a certain threshold sulfate concentration; and (3) a slope at linearity less than the Nernstian value, but sufficiently large and reproducible to be useful analytically.

In Table 1, the potential shift, the threshold concentration, and the linear slope are given for three types of electrodes in a variety of background electrolytes. Inclusion of either barium sulfate or 2-AP sulfate substantially improved selectivity over nitrate. The selectivity over chloride was only slightly affected by solids added to the membrane. The ratio of sulfate to background electrolyte concentration at the beginning of linear response is also given in Table 1. From these ratios, a selectivity series for the 2-AP electrode for monovalent ions was established: the order of interference was

$$ClO_4^- >> Cl^- > NO_3^- > H_2PO_4^- > F^-.$$

The effect of pH. The effect of pH is shown in Fig. 2; the ordinate is the shift in potential reading from that at pH 5.3, the observed pH of the Na_2SO_4 solutions. One set of symbols in Fig. 2 represents an experiment in which the pH of 10^{-2} M Na_2SO_4 was adjusted by adding either HCl or NaOH. In the other set, adding the foreign anion was avoided by neutralizing 10^{-2} M H_2SO_4 with NaOH. The general trend of response is the same for both experiments. The useful pH range at 10^{-2} M sulfate is 4—9.

TABLE 1

Effect of other electrolytes on sulfate electrode response for electrodes with and without $BaSO_4$ or 2-AP

Background electrolyte	No Additive				$BaSO_4$				2-AP			
	Potential shift (mV)	"Threshold" SO_4^{2-} concn. (M)	Slope (mV)	SO_4^{2-}: X ratio[a]	Potential shift (mV)	"Threshold" SO_4^{2-} concn. (M)	Slope (mV)	SO_4^{2-}: X ratio	Potential shift (mV)	"Threshold" SO_4^{2-} concn. (M)	Slope (mV)	SO_4^{2-}: X ratio
None	0	3×10^{-5}	29	—	0	$\sim 2 \times 10^{-5}$	30	—	0	2×10^{-5}	30	—
10^{-4} M $NaNO_3$	−14	5×10^{-4}	21	5	—	—	—	—	−30	5×10^{-5}	30	0.5
10^{-3} M $NaNO_3$	−82	$>10^{-3}$	—	—	—	—	—	—	−70	2.5×10^{-4}	19	0.25
10^{-3} M NaCl	−17	7×10^{-4}	26	0.07	−38	2.5×10^{-4}	24	0.25	−40	2.5×10^{-4}	24	0.25
10^{-2} M NaCl	—	—	—	—	—	—	—	—	−80	2×10^{-3}	16	0.2
10^{-4} M $KClO_4$	—	—	—	—	—	—	—	—	—	2×10^{-4}	23	2
10^{-3} M NaH_2PO_4 (pH 5.2)	—	—	—	—	—	—	—	—	−48	2×10^{-4}	26	0.2
10^{-2} M KF	—	—	—	—	—	—	—	—	−48	2×10^{-4}	24	0.02
10^{-3} M Na_2HPO_4 (pH 8.3)	—	—	—	—	—	—	—	—	−57	2×10^{-3}	25	2

[a]Where X is the background electrolyte.

Behavior in use. For a good electrode, the value of the potential in pure water increased with repeated changes to fresh water, until a fairly reproducible value (±2 mV) was reached. This phenomenon probably corresponds to leaching of sulfate from the electrode. Sensitivity of the electrode was increased by successive immersion in fresh water, after which real (but not Nernstian) response could be observed at sulfate concentrations below 10^{-5} M. The response of the electrode at steady state in solution to sulfate additions was almost instantaneous; readings were steady to ±0.2 mV.

One continuing problem was eventual deterioration of the membrane with continued service in aqueous solutions. After a week or so of daily use, the membrane became chalky, adherence to the platinum surface diminished, and the observed potential reading drifted steadily downward. Presumably, this deterioration was caused by the leaching of solvent from the membrane. Binding the edges of the electrode with "Parafilm" retarded electrode failure, but did not completely eliminate the problem.

Standard potential values of all electrodes were similar, but shifted 1—2 mV from day to day. An ultimate downward trend culminated in the failure of the electrode, as described above.

Analytical techniques

Because foreign ions shift the potential of the electrode, even though response to sulfate is retained, determination of sulfate by direct comparison of sample and standard may not always be suitable. However, the standard addition method [10] is practical, particularly because the electrode works best when sulfate is added to the solution in situ, rather than when the electrode is transferred from one solution to another. If the slope of the response is in doubt, more than one addition can be made to eliminate the necessity of assuming a value for the slope [11]. Alternatively, the addition/dilution technique [12] can be used to detect a major interference, which will be indicated by an abnormally large potential change on dilution.

Attempts to use the electrode as indicator in potentiometric titrations (e.g., SO_4^{2-} with $BaCl_2$) failed because of the interference of the anion introduced with the titrant. Titrations with an initial background of chloride were only marginally acceptable.

The electrode has been used to determine the sulfate content of various water samples by the standard addition method. The usefulness of the electrode has been the ease of fabrication, which provides a working electrode when required, and the simplicity of the attendant analytical procedures.

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DIFFERENTIAL PULSE POLAROGRAPHIC DETERMINATION OF HYDROCORTISONE IN PHARMACEUTICAL PREPARATIONS

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SUMMARY

Differential pulse polarograms of hydrocortisone recorded from acetate buffer pH 4.6 exhibit a well-defined peak at −1.25 V vs. SCE and the peak current is proportional to the concentration in the range 10^{-5}—8×10^{-5} M. The electrode reaction involves one electron and one hydrogen ion. A simple rapid method is proposed for the determination of hydrocortisone in creams and ointments. The procedure does not involve time-consuming extractions, but it is not applicable to samples containing surfactants like polyethyleneglycol which are more strongly adsorbed on the electrode than hydrocortisone. Because degradation in the side chain of hydrocortisone is not detected polarographically, the method is suitable for production control but not for stability tests.

Hydrocortisone is usually determined by colorimetric [1—5] or chromatographic [6—8] methods. The drug is reduced at the dropping mercury electrode, and can also be determined by d.c. polarography [9—11]. The determination of hydrocortisone in complex formulations like creams and ointments is very time-consuming because preliminary multi-step extraction techniques are usually required. The object of the present work was to study the electroreduction of hydrocortisone and to investigate the application of differential pulse polarography to faster and simpler analyses for the drug in creams and ointments.

EXPERIMENTAL

Instrumentation

All polarograms were recorded with a Princeton Applied Research Model 174 Polarographic Analyzer connected to a Houston Omnigraph 2000 XY recorder. Conventional types of dropping mercury electrode and of electrolysis cell were used. The capillary characteristics measured in 0.1 M acetate buffer pH 4.6 at a corrected mercury height of 67.8 cm were $m = 3.578$ m gs^{-1} and $t = 2.46$ s. Cyclic voltammetry was done with a versatile solid-state instrument constructed to the design of Goolsby and Sawyer [12]. A Moosley 7030 AM XY recorder was used with this instrument, and a Metrohm E 410 hanging mercury drop was used as working electrode. A PAR saturated calomel

electrode (SCE) was used as reference electrode and a platinum coil served as auxiliary electrode in all experiments. Dissolved air was removed from the solutions by bubbling oxygen-free nitrogen through the cell for 5 min and passing it over the solution during the electrolysis. All experiments were performed at 25 ± 0.1°C.

Materials

Hydrocortisone (pharmaceutical grade), Milliderm cream A.L. (containing 0.1% hydrocortisone in an oil/water cream base), and Salvizol ointment with hydrocortisone A.L. (containing 1% hydrocortisone and 0.4% dequalinium chloride in a water/oil ointment base) were obtained from A/S Apothekernes Laboratorium for Specialpraeparater (A.L.), Oslo.

A 1-mM stock solution of the drug was prepared by dissolving 0.36 g of hydrocortisone in 50 ml of ethanol and diluting to 1 l with distilled water. Acetate buffer pH 4.6 (0.1M) was prepared by dissolving 6.00 g of acetic acid and 8.20 g of sodium acetate in distilled water and diluting to 1 l. All other chemicals were of reagent grade and used without further purification.

RESULTS AND DISCUSSION

Differential pulse polarographic behaviour of hydrocortisone

Differential pulse polarograms of 10^{-5} M hydrocortisone recorded from strongly acidic media exhibit only a tensammetric wave at 0 V vs. SCE. When the pH of the electrolyte was increased above 3.5, a single reduction wave appeared at potentials close to the reduction wave of the supporting electrolyte. In the pH range 4—6, the wave was well defined, but a further increase in pH resulted in a splitting of the wave and a large decrease in the peak current (Fig. 1).

Many ketosteroids have previously been determined with 10—50% ethanol as solvent [13]. Experiments showed, however, that addition of ethanol (20—40%) to the supporting electrolyte resulted in a splitting of the single wave observed at pH 4—6. Above pH 10 a single well-defined wave was observed in the presence of ethanol, but the peak height was independent of the hydrocortisone concentration. It was shown that this wave is a negative tensammetric wave. Obviously, the presence of ethanol has no advantage in the polarographic determination of hydrocortisone and in the following experiments the ethanol content was kept as low as possible.

Several years ago, Zuman et al. [13] investigated the electroreduction of desoxycorticosterone (which has almost the same chemical structure as hydrocortisone). They claimed that the reduction wave observed below pH 6 is a catalytic hydrogen wave. Since the present experiments showed that the best-defined wave is obtained in the pH range 4—6, the electrode reaction of hydrocortisone in this pH range was examined in more detail in order to verify the nature of the wave.

The effect of drop time was investigated by recording d.c. polarographic

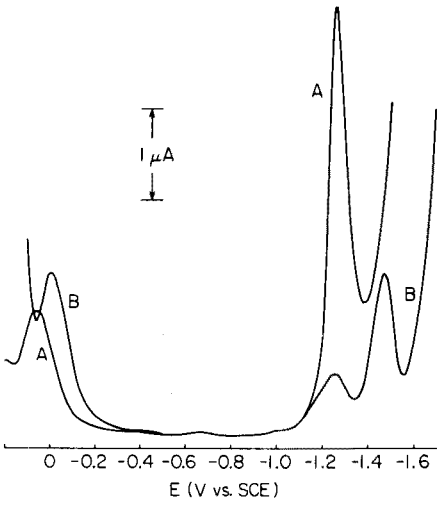


Fig. 1. Differential pulse polarogram of 5×10^{-5} M hydrocortisone in acetate buffer pH 4.6 (curve A) and in phosphate buffer pH 6.7 (curve B). Drop time 1 s, scan rate 5 mV s^{-1} and pulse amplitude 50 mV.

curves of 5×10^{-5} M hydrocortisone in acetate buffer pH 4.6 at various heights of the mercury column. The value $i\,h^{-\frac{1}{2}}$, where h is the height of the column after correction for the "back pressure", was constant, indicating that the current is diffusion-controlled. The temperature coefficient (determined in the range 20—45°C) of the d.c. wave was +1.9% per degree; this again implies that the current is essentially controlled by diffusion.

The tensammetric wave observed on the differential pulse polarograms indicates that the drug is adsorbed on the electrode surface. Adsorption was verified by drop time measurements. As indicated in Fig. 2, the presence of hydrocortisone causes a large decrease in the drop time over a considerable potential range, indicating that the drug is surface-active and is strongly adsorbed on the electrode in the entire potential range +0.2 to −1.4 V.

The effect of pH on the current—voltage curves was investigated by recording differential pulse polarograms of 5×10^{-5} M hydrocortisone in phthalate, citrate, acetate, phosphate and borate buffers. The best-defined waves were obtained from acetate buffers. The peak potential was shifted to more negative values with increasing pH of the electrolyte, indicating that hydrogen ions are involved in the electrode reaction. As indicated in Table 1, the peak current decreases rapidly with increasing pH of the electrolyte. Consequently, a buffer of high capacity is required; in all the following experiments, a 0.1 M acetate buffer of pH 4.6 was used as supporting electrolyte.

Experiments showed that well-defined polarograms and relatively high peak currents were obtained when a drop time of 1 s, scan rate 2 mV s^{-1}, and pulse amplitude 50 mV were used. Because the reduction of hydrocortisone occurs at high negative potentials and the reduction of hydrogen ions partly interferes

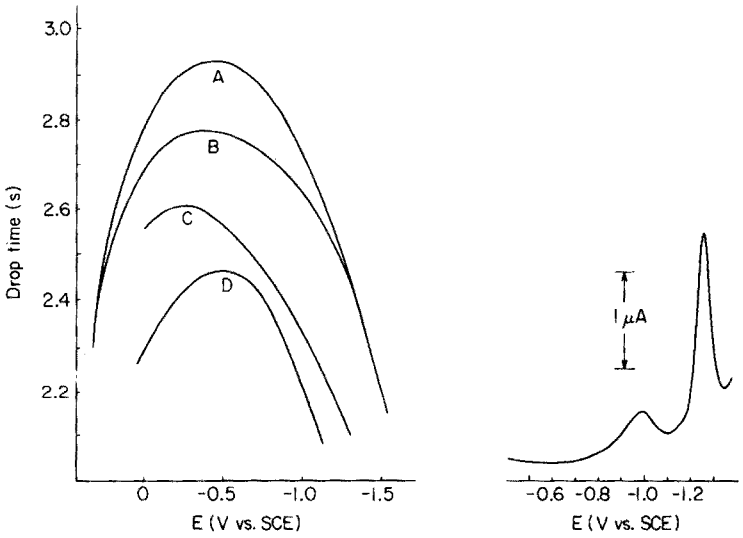


Fig. 2. Electrocapillary curves in 0.1 M acetate buffer pH 4.6 (curve A) and in the presence of 7.5×10^{-5} M hydrocortisone (curve B), 0.02% macrogol 400 (curve C) and 0.001% dequalinium chloride (curve D).

Fig. 3. Differential pulse polarogram of 1.08 g of Milliderm cream A.L. per 100 ml in acetate buffer pH 4.6. Drop time 1 s, scan rate 2 mV s^{-1} and pulse amplitude 50 mV.

at low concentrations of hydrocortisone, the most reproducible results were obtained when the current was measured from the foot of the wave rather than by the "base-line" method.

Polarograms recorded from acetate buffer pH 4.6 with various amounts of hydrocortisone present, showed that the peak current increased linearly with concentration in the range 10^{-5}—8×10^{-5} M. At lower concentrations interference from the reduction of hydrogen ions was observed and at higher

TABLE 1

Effect of pH on differential pulse polarograms of 5×10^{-5} M hydrocortisone (Drop time 1 s, pulse amplitude 50 mV, and scan rate 5 mV s^{-1}.)

Buffer	pH	$-E_p$ (V)		i_p (μA)	
Acetate	3.7	1.21		7.55	
	4.3	1.22		4.92	
	4.6	1.25		4.70	
	5.1	1.29		3.43	
Phosphate	5.9	1.37		1.87	
	6.7	1.25	1.47	0.68	1.81
	7.7	1.28	1.66	0.67	1.58
Borate	8.7	1.32	1.67	0.63	1.58

concentrations the standard curve was slightly curved, probably because of the strong adsorption on the electrode. The diffusion current constant calculated from the d.c. current was $I = 1.44\ \mu A\ mM^{-1}\ mg^{-\frac{2}{3}}s^{\frac{1}{2}}$, which indicates that only one electron is involved in the electrode reaction. The stock solution of hydrocortisone, as well as standard solutions prepared in 0.1 M acetate buffer, were polarographically stable. No decrease in peak current was observed 3 weeks after mixing the solutions.

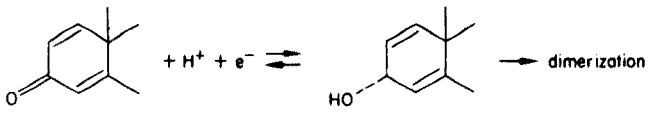
Voltammetric experiments were done at a hanging mercury drop electrode. Reproducible results were obtained provided that the mercury drop was changed between each experiment and just before the next potential sweep. Voltammograms recorded from acetate buffer showed a cathodic peak at potentials corresponding to the polarographic step. A small anodic peak resulting from re-oxidation of the reduction product was observed at high scan rates, indicating a reversible step in the overall electrode reaction. The difference between the cathodic and the anodic peak potential, $\Delta E = 0.06$ V, indicates a one-electron reduction. No anodic peak was observed at slow scan rates, indicating that the reversible step is followed by an irreversible chemical reaction.

The current function, $i_p/C\nu^{\frac{1}{2}}$, appeared to be very dependent on both the scan rate and the bulk concentration, but it did not increase with decreasing scan rate as in the case of a catalytic electrode reaction [14]. On the contrary, the current function increased rapidly with increasing scan rate and decreasing concentration. At slow scan rates and high bulk concentrations the current function approached a constant "diffusion-controlled" value. This is a characteristic feature for surface-active depolarizers [15]. At sufficiently fast scan rates, the amount of diffusing material is small compared to the amount adsorbed at the electrode surface. In contrast, when the bulk concentration is increased, the adsorption has a lesser relative influence on the peak current.

It is evident from the data given above that the polarographic wave obtained from acetate buffer pH 4.6 is a one-electron reduction wave of hydrocortisone and that the depolarizer is strongly adsorbed on the electrode surface. The number of hydrogen ions, Z, involved in the electrode reaction is given by:

$$\Delta E_{\frac{1}{2}}/\Delta pH = -0.059 \cdot Z/\alpha n_a$$

where α is the transfer coefficient and n_a the number of electrons in the rate-controlling step. Inserting the shift of the peak potential with increasing pH (—105 mV per pH unit) into this equation and assuming that $\alpha = 0.5$, gives the value $Z = 0.9$, which indicates that one hydrogen ion is involved. The voltammetric experiments indicate a reversible one-electron reduction which is followed by an irreversible chemical reaction. Hence, the most probable electrode reaction is reduction of the keto group to a free radical which dimerizes and irreversibly forms a pinacol



This reaction path has previously been suggested for other ketosteroids [16].

The reactivity of the side chain of the ketosteroids has been well documented [17, 18]. In the presence of air an oxidative cleavage of the side chain to yield the corresponding etianic acid has been reported; even in the absence of air decomposition to hydroxy acid may occur. Such degradative processes in the side chain are not observed polarographically. Hence, the present method is not applicable for the determination of decomposition or stability analysis of hydrocortisone.

Analytical applications

Differential pulse polarographic determinations of drugs in creams and ointments are usually not possible because the waves are greatly distorted by the adsorbed film of surfactants present in such materials. However, the strong adsorption of hydrocortisone and the high negative reduction potential indicate that the drug might be determined in the presence of surfactants without prior separations [19].

Creams and ointments are not readily soluble in acetate buffer. However, hydrocortisone could be quantitatively extracted when the sample was heated to 60°C in ethanol before addition of the supporting electrolyte. Following this procedure, a well-defined hydrocortisone wave was obtained from Milliderm cream A.L., which is a new type of hydrocortisone cream containing only 0.1% dissolved hydrocortisone. The surfactants present in this cream caused a decrease of about 16% in the peak current but the standard curve was still linear in the concentration range 10^{-5}—8×10^{-5} M. Based on these experiments the following procedure is suggested.

Recommended procedure. Transfer about 1 g of cream (containing 0.1% hydrocortisone) to a weighing bottle with glass lid. Add 1.0 ml of ethanol and heat the suspension at 60°C for about 30 min. Transfer the suspension to a 100-ml flask and dilute to 100 ml with 0.1 M acetate buffer pH 4.6. Transfer a suitable amount of the suspension to a polarographic cell, deaerate with pure nitrogen, and record a differential pulse polarogram with starting potential —0.5 V, scan rate 2 mV s^{-1}, pulse amplitude 50 mV and drop time 1 s. Measure the peak current from the foot of the wave and determine the concentration from a calibration curve obtained by the same procedure and in the presence of the same amount of cream base.

A polarogram of hydrocortisone in Milliderm cream obtained by the above procedure is given in Fig. 3. The peak at —1 V is a negative tensammetric peak resulting from desorption of dodecyl sulphate. The peak current of hydrocortisone is decreased by the presence of such surfactants, and the standard curve must be prepared with the same amount of cream base. The surfactants in the cream also cause foaming during the deaeration, but much less foam is obtained if the suspension is left in the flask for 1—2 h with occasional shaking before transferring an aliquot to the cell.

The results of a few determinations of hydrocortisone in Milliderm cream A.L. are given in Table 2. The relative standard deviation of 5 samples was 4.3%.

TABLE 2

Polarographic determination of hydrocortisone in Milliderm cream A.L., lot no 8/75. (Declared amount of hydrocortisone 0.1%)

Amount of cream (g/100 ml)	Current (μA)	Hydrocortisone found (mM)	% Hydrocortisone
1.1301	2.470	0.0314	0.101
1.5053	3.285	0.0409	0.099
0.7534	1.520	0.0203	0.098
1.1290	2.680	0.0339	0.109
1.5053	3.460	0.0430	0.104
		Mean 0.102 ± 0.004%	

The above procedure was also used in an attempt to determine hydrocortisone in Salvizol with hydrocortisone A.L. ointment. However, a hydrocortisone peak was not observed. Dequalinium chloride and polyethyleneglycol (macrogol 400 and 3000) which are present in this ointment base, are more strongly adsorbed on the electrode than hydrocortisone (Fig. 2) and are desorbed at potentials much more negative than the reduction potential of hydrocortisone. Moreover, the adsorbed film has a very compact structure, so that reduction of hydrocortisone is completely inhibited, and prior separation is necessary in this case.

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A SIMPLE ROUTINE METHOD FOR THE RAPID DETERMINATION OF ORGANIC AND INORGANIC CARBON IN OIL SHALE

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SUMMARY

A procedure for the rapid determination of organic and inorganic carbon in oil shale samples is proposed. Oil shale samples are decomposed in an oxygen stream at three different temperatures (450°C, 550°C, 900°C). The resulting CO_2 is determined after absorption in 0.02 M NaOH in a relative conductometric detection unit. Temperature-differentiated carbon analysis was used to establish the decomposition temperatures of the organic material (450°C) and the inorganic fractions (550°C and 900°C). The method was tested for samples weighing 2—4 mg. Oil shales with organic carbon contents of 8—20% were determined with good reproducibility (r.s.d. 0.4—1.3%). The accuracy was tested with a standard oil shale sample. One determination requires 8 min.

Although the potential economic importance of oil shale was reported nearly sixty years ago [1], only after the current energy shortages has any significant effort been made to exploit the material. Compared to other fossil fuels such as petroleum and coal, the organic matter in oil shale is diluted, but estimates indicate that the absolute abundance of the diluted fossil fuels exceeds the concentrated by three orders of magnitude. Yen [2] points out that slightly more than 5% of the sedimentary rocks in the USA are shales containing more than 5% organic material. Since sediments cover the continents to an extent of 90%, these rocks play an important part in the world energy reserve. Applying current technology, "The Oil Shale Corporation" (TOSCO) estimates the production costs of crude oil [3]. Because the oil yield of shales can vary in the range 0—200 gallons/ton, quick tests to determine the organic content of the shale are required for prospecting and evaluation of potential deposits.

The most important method for the determination of the oil yield is the Fischer assay, which is a carefully controlled analytical distillation of the shale coming close to simulation of the industrial retorting process [4]. Since the oil yield of a shale is related to the organic carbon content, several methods for its estimation are based on direct or indirect determination of the organic components. Frost and Stanfield [5] and Smith [6]

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estimated the content of organics by determining the relationship between specific gravity of the shale and the volume of the organic matter. Wide-line nuclear magnetic resonance [7] and pulsed nuclear magnetic resonance [8] were used to estimate the organic content via the resonant protons, the latter technique giving the results in 2 min. Recently, proton-enhanced carbon-13 nuclear induction spectrometry was applied to estimate the composition of the kerogen in oil shales from the Green River formation [9]. Temperature-programmed heating of oil shales in a helium stream and gas analysis of the volatilized organics by flame ionization detection (f.i.d.) allows an estimate of the recoverable oil yield since a correlation between f.i.d. peak areas and Fischer assay data could be established [10]. Fast laser pyrolysis and gas-chromatographic separation with quantitative evaluation of the low-molecular-weight gaseous products (H_2, CO, C_2H_2, C_2H_4 and H_2O) can be used to calculate water, oil, organic carbon, organic hydrogen and carbonate content of the sample [11]. A major advantage of elemental analytical techniques is the capability of precise and accurate determination of certain elements. Extensive studies were carried out by Cook [12] whose method is now widely used. The organic carbon is determined indirectly by calculating the difference between total carbon and carbonate carbon. Simple but very effective is the direct determination of the "organic carbon" by controlled combustion as reported by Heistand and Humphries [13]. The correlation between oil yield as determined by the Fischer assay and the direct "organic carbon" values indicated the reliability of this method. Recently, a method for speciation of carbonaceous material in dust samples was reported by Malissa et al. [14] who used a relative-conductimetric device as the evolved gas detector. Organic and inorganic carbon species can be separated because of their different pyrolytic behavior. This method was applied to determine the content of organic and inorganic carbon in oil shale.

EXPERIMENTAL

The principles and apparatus for relative-conductimetric elemental analysis [15] and for temperature-differentiated carbon analysis [14] have already been described. The sample is heated in a tube furnace in an oxygen stream. The combustion products pass through an absorption tube filled with silver wool at 450°C to remove SO_2 and halogens, and enter the absorption cell of the automatic device "Carmhomat" (H. Woesthoff OHG) where the CO_2 is absorbed in 0.02 M NaOH. The conductivity is converted to a mV-signal by means of a bridge circuit and is recorded on a strip-chart recorder. This signal is proportional to the amount of CO_2 released.

A series of experiments were carried out by slow decomposition of oil shale samples in an oxygen stream; the linear temperature program used had a heating rate of 12°C min^{-1}. A typical record of the CO_2 evolution during the heating process is given in Fig. 1. The decomposition of the organic

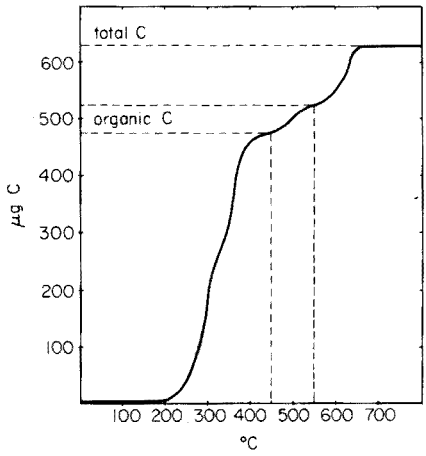

Fig. 1. Temperature-programmed run of oil shale sample OS 1. A 3-mg sample was used with a programmed temperature increase of 12°C min^{-1}.

material starts at 200°C, shows two reaction-rate maxima at 300 and 350°C, when investigated in detail, and ends at 450°C. Second and third steps caused by the decomposition of inorganic carbonates appear with maximal reaction rates at 530 and 650°C. From these tests, the decomposition temperature for the direct organic carbon determination was established as 450°C. Furthermore, the amount of inorganic carbon yielded at 450—550°C allows an estimate of carbonates which may decompose during a retorting process. The inorganic carbon at 550—900°C is due to the decomposition of the more stable carbonates, mainly calcite and dolomite.

The apparatus was calibrated by decomposing $CaCO_3$ samples containing 100—400 µg C at 900°C. Linear regression analysis yielded eqn. (1) with a correlation coefficient of 0.9998:

$$y = 1.30\ (\pm 0.01)\, x\ [\mu g\ C] - 1.7\ (\pm 2.7) \quad (1)$$

(y is the instrument readout in mV and x the carbon content of the sample in µg).

For the direct determination of organic and inorganic carbon in oil shale, a finely ground oil shale sample (2—4 mg) is weighed into a porcelain boat and spread to a thin layer, and the boat is placed in the furnace at a pre-set temperature. According to the results of the temperature-differentiated detection of carbon in oil shale samples, three temperatures (450, 550 and 900°C) were chosen for carrying out direct carbon determinations. The pyrolysis is complete within 6 min. Filling and reset of the apparatus requires 2 min, so that the total analysis time for one sample is 8 min.

RESULTS AND DISCUSSION

Repeated runs of an oil shale sample at the three temperatures mentioned indicate that the three different carbon fractions can be determined with good reproducibility (Table 1). The accuracy was tested with a standard sample. Table 2 shows excellent agreement between the standard and conductimetrically determined values. A comparison of the carbon values obtained by the temperature-differentiated method [14] (slow heating) and direct carbon determination (fast combustion) is shown in Table 3. The agreement of these results (all of which are the mean of triplicate measurements) indicates that the exothermic oxidation process of the organic material does not affect the decomposition of the carbonates to a

TABLE 1

Repeated runs of sample OS 8

T (°C)	n	Carbon (%)		R.s.d. (%)
		$\bar{x}$	S.d.	
900	7	18.33	0.072	0.39
550	6	16.04	0.213	1.33
450	7	15.51	0.143	0.92

TABLE 2

Comparison between standard and relative conductimetric values

Paraho Standard Raw Shale	Standard value	Rel. cond. value
Total carbon (%)	17.50	17.52 ± 0.3
Organic carbon (%)	12.70	12.80 ± 0.1
Oil Yield (gal./ton)	29.2	29.5[a]

[a]Calculated from eqn. (2).

TABLE 3

Comparison between values for temperature-differentiated carbon analysis and direct carbon analysis for sample OS 1

Temperature range (°C)	%C[a]	%C (direct)
23—450	16.0	15.9
450—550	1.5	1.7
550—900	3.5	3.6
Total %C	21.0 ± 0.2	21.2 ± 0.2

[a]From temperature-differentiated carbon detection.

significant extent. Table 4 shows results for several samples of a depth profile of oil shales from a Wyoming site in the Green River formation, which were chosen to demonstrate the applicability of the relative conductimetric method. As the oil yield from oil shale is almost entirely a function of the kerogen content, which can be estimated by determining the percentage of organic carbon [16], the correlation between organic carbon content and oil yield of shales of the Green River formation is now an established function [12, 13]. For the calculation of the oil yield shown in Table 2, the equation applied was

$$\text{Oil (gal/ton)} = 2.37 \times \text{org. C(wt.\%)} - 0.80 \quad (2)$$

The amount of the organic matter was obtained by multiplying the organic carbon value by the conversion factor 1.24 [17]. The sum of the inorganic carbon fractions I and II is also expressed as total inorganic CO_2.

Limitations of the "direct organic carbon" determination will occur, when Dawsonite or Nahcolite, two minerals which dissociate partially below 450°C, are present in the sample. Nahcolite dissociates at 170°C, yielding 0.5 mol of CO_2. Dawsonite decomposes around 350°C also giving 0.5 mol of CO_2 [18]. However, Dawsonite is very rare and the only known occurrence in large quantity is restricted to a well known area in Colorado [18, 19]. Nahcolite occurs more frequently, usually in minor amounts, but it is enriched in the Dawsonite-bearing ores. Therefore in most cases no significant influence of these minerals on the "direct organic carbon" value will occur [13]. Considering a medium-rich shale containing 15% organic carbon, a content of 3% Dawsonite will yield an error of an additional 0.12% C, whereas 2% Nahcolite will give an additional 0.14% C to the true organic value; these errors are below 1% relative of the organic carbon value.

Conclusions

Considering the time required for a relative conductimetric carbon determination (8 min), the precision and the accuracy of the direct organic

TABLE 4

Direct determination of inorganic and organic carbon in oil shale samples

OS	Sample	Direct carbon values (%C)			Organic matter (wt %)	Inorganic C"I" (450—550°C) (%C)	Inorganic C"II" (550—900°C) (%C)	Total inorganic CO_2 (%CO_2)
		900°C[a]	550°C	450°C[b]				
1	183'8"B	21.2	17.6	15.9	19.7	1.7	3.6	19.4
2	183'8"G	22.3	19.2	18.1	22.4	1.1	3.1	15.4
3	192'8"E	11.1	9.3	8.6	10.7	0.7	1.8	9.2
4	192'8"E	11.9	10.0	9.8	12.2	0.2	1.9	7.7
5	201'4"D	17.0	15.2	14.6	18.1	0.6	1.8	8.8
6	201'4"D	14.7	13.4	13.1	16.2	0.3	1.3	5.9
7	208'8"B	13.0	11.1	10.6	13.1	0.5	1.9	8.8
8	208'8"D	18.3	16.0	15.5	19.2	0.5	2.3	10.3

[a] "Total carbon". [b] "Organic carbon".

carbon value, the method is superior to non-destructive techniques based on specific gravity [6] or n.m.r., [7—9] as well as techniques based on pyrolysis in inert gas streams [10, 11]. Compared to the indirect [12] and the direct [13] organic carbon determination methods presently used, considerable time is saved.

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AN AUTOMATED PROCEDURE FOR THE SIMULTANEOUS DETERMINATION OF SPECIFIC CONDUCTANCE AND pH IN NATURAL WATER SAMPLES

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SUMMARY

An automated, continuous-flow system is utilized to determine specific conductance and pH simultaneously in natural waters. A direct electrometric procedure is used to determine values in the range pH 4—9. The specific conductance measurements are made with an electronically modified, commercially available conductivity meter interfaced to a separate module containing the readout control devices and printer. The system is designed to switch ranges automatically to accommodate optimum analysis of widely varying conductances ranging from a few μmhos cm^{-1} to 15,000 μmho cm^{-1}. Thirty samples per hour can be analyzed. Comparison of manual and automated procedures for 40 samples showed that the average differences were 1.3% for specific conductance and 0.07 units for pH. The relative standard deviation for 25 replicate values for each of five samples was significantly less than 1% for the specific conductance determination; the standard deviation for the pH determination was $\leq$ 0.06 pH units.

Specific conductance and pH determinations are commonly used as indicators of water quality. Manual procedures are usually employed [1] although an automated method involving a minicomputer has been reported for determining pH, specific conductance, and water hardness at a rate of 20 samples per hour [2]. For a laboratory to make optimum use of automated determinations for pH and specific conductance, it would be highly desirable to increase that sampling rate and be able to analyze samples routinely with specific conductances as high as 15,000 μmho cm^{-1}.

A relatively simple, continuous-flow system was therefore designed to determine pH and specific conductance simultaneously while minimizing personnel and sample volume requirements. Natural waters have widely varying specific conductances; therefore, it was also necessary to incorporate an automatic range switching device into the system by modification of commercially available instrumentation. With this feature, it became possible to determine conductances automatically in the range 1—15,000 μmho cm^{-1} with a precision that is generally less than 1% of the mean value. The pH was determined electrometrically in the range 4—9 [3].

EXPERIMENTAL

Apparatus and reagents

The following Technicon AAII modules were used for measuring pH: Sampler IV with 30(6/1) cam, proportioning pump III, ion-selective electrode module (I.S.E.), and pH combination, flow-through electrode, recorder, and printer. A Radiometer CDC314 flow-through conductance cell is connected in tandem with the pH electrode (Fig. 1) and is also housed in the thermostatically-controlled compartment of the electrode module. The platinum wire protruding into the chamber of the pH electrode permits the use of an air-segmented sample stream without the potentiometric fluctuations which normally would occur if continuous contact were not made between the sample segments. The temperature should be somewhat higher than that normally encountered in the laboratory; in this case it was regulated at 30°C. In addition to the applicable Technicon modules, the instrumentation for determining specific conductance includes: (1) a Radiometer CDM3 specific conductance meter with a Model CDA100 temperature compensator and custom-built circuitry; and (2) an assembly of DigiTec electronic modules consisting of (a) two Model 2780N d.c. panel meters (4 1/2 digits), one with a Model 2785 range adapter; (b) a Model 8130ML programmable factoring timer; (c) two Model 685 digital comparators; and (d) Model 6150 printer modified to delete the internal clock printout.

The timing assembly (comparator and factoring timer) triggers the printer at the appropriate time. This time interval is adjustable and set at 120 s in the present application. The other comparator establishes a preset level for actuating the range switch circuit. The lower range digital voltmeter, DVM 1, is internally calibrated so that a full scale reading of the conductivity meter (1 V d.c.) represents 1,500 μmho cm^{-1}. If the meter reading is on scale (the output of the voltmeter is below the limit set on the range comparator), then the output of DVM 1 is transferred to the printer where it is recorded when triggered by the timing assembly. If the value of the sample reading is greater than the limit set on the range comparator, the switching circuit is initiated and the range is switched to the next higher decade (0—15,000 μmho cm^{-1} range) by a relay mechanism. The circuit also disengages DVM 1

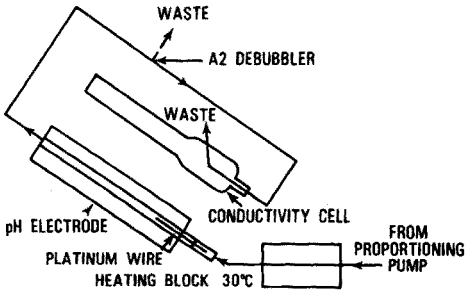


Fig. 1. I.S.E. compartment arrangement.

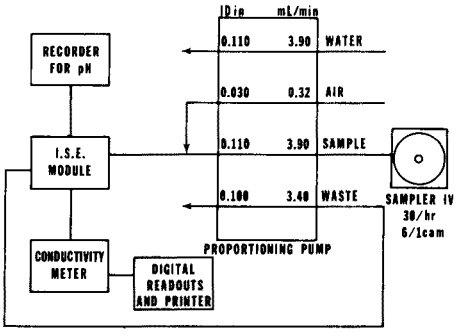
and the output from DVM 2 is recorded when the printer is triggered. After printing, the switching mechanism automatically resets to the lower scale and DVM 1 is activated for the next sample. Additional information concerning the electronics of this system may be obtained elsewhere [4].

A flow diagram of the system and a block diagram of the interconnection of the components are shown in Figs. 2 and 3, respectively.

All reagents were prepared in demineralized water. Reagent grade KCl was used for the preparation of 0.00702 M, 0.0200 M, and 0.100 M solutions which have specific conductances of 1,000, 2,767, and 12,900 μmho cm^{-1}, respectively [1, 5]. Commercially available buffer solutions, pH 4 and pH 7, were used.

After the temperature has stabilized in the thermostated compartment of the electrode module, adjustment is made with the temperature control on the conductivity meter to give a reading of 1,000 μmho cm^{-1} on DVM 1 with 0.00702 M KCl. In addition, offset and potentiometer adjustments are provided to allow calibration of DVM 2. The slope adjustment is set, after the switching mechanism has been activated, to give a reading of approximately 12,900 μmho cm^{-1} with the high standard (0.100 M KCl). The offset control is then adjusted to give a reading of approximately 2,767 μmho cm^{-1} for the 0.0200 M KCl solution. This procedure is repeated until both the high and intermediate standards read correctly. Calibration in this manner automatically provides all readings in the units commonly used, i.e. μmho cm^{-1} at 25°C.

The pH calibration procedure is similar. For the range pH 4—9, the baseline and STD CAL controls on the electrode module are adjusted to give readings of 0 and 60 scale divisions on the recorder when buffers of pH 4 and pH 7, respectively, are introduced into the system. This procedure of alternating buffers and making control adjustments is repeated until both buffer solutions read correctly.



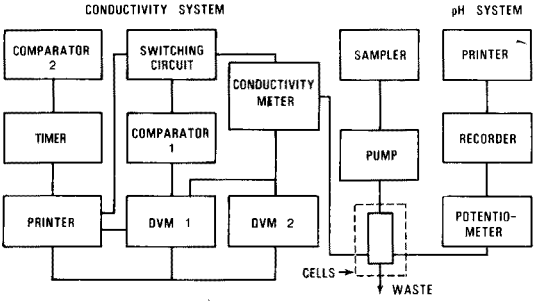


Fig. 2. Flow diagram for the determination of specific conductance and pH.

Fig. 3. Block diagram depicting arrangement of individual modules in the automated system.

RESULTS AND DISCUSSION

Replicates from each of five samples were randomly placed in a sample tray and both specific conductance and pH were determined over a 5-day period. The positioning of the samples varied from day to day to remove any possible bias from sample order. Table 1 shows that the relative standard deviations range from 0.3% for sample 1, to 0.8% for sample 4. The pH results were also precise, with a maximum standard deviation of 0.06.

Results of this automated procedure were compared with manual methods [1] for both specific conductance and pH. Forty natural water samples were analyzed. The overall average differences between the procedures was 1.3% for the specific conductance determination and 0.07 units for the pH values. In addition to the excellent agreement between procedures, there is no evidence of any positive or negative bias. The results from 15 randomly selected samples are listed in Table 2.

A carryover problem may arise in the specific conductance determination if a sample with comparatively low-conductance is preceded by one with much higher conductance. The results of a carryover study are shown in Table 3. The samples were analyzed in the order listed. The data show that a sample with a conductance of ca. 100 μmho cm^{-1} can be preceded by one with a conductance of up to 7,500 μmho cm^{-1} and the resulting carryover will not exceed 5%. If the specific conductance of a sample is between 7,500 and 10,000 μmho cm^{-1}, the carryover becomes significant for the next sample if its conductance is less than 5% of that of the first. Likewise, if the specific conductance of a sample is between 10,000 and 15,000 μmho cm^{-1}, the carryover becomes significant for the next sample if its conductance is les than 10% of that of the first. These conditions are not encountered frequently in natural waters, but they should be considered as guidelines if it is necessary to re-analyze a sample because of carryover from the preceding sample.

The sample stream passing through the pH electrode is segmented with air bubbles and the volume of the cell is small; carryover from the previous sample is not a significant problem.

TABLE 1

Five-day precision study for specific conductance and pH

Sample[a]	Specific conductance (μmho cm^{-1})		pH	
	Range	Mean ± 1 s.d.	Range	Mean ± 1 s.d.
1	682 — 690	685.6 ± 2.2	7.45 — 7.68	7.58 ± 0.05
2	305 — 311	307.4 ± 1.6	7.98 — 8.13	8.07 ± 0.03
3	1,645 — 1,679	1,664 ± 11	7.48 — 7.63	7.58 ± 0.04
4	95 — 98	96.9 ± 0.8	7.48 — 7.83	7.69 ± 0.06
5	811 — 822	815.4 ± 3.0	8.09 — 8.20	8.14 ± 0.03

[a] 25 replicates of each sample taken.

TABLE 2

A comparison of manual and automated results for specific conductance and pH determinations

Sample	Specific conductance (μmho cm^{-1} at 25°C)		pH	
	Manual	Automated	Manual	Automated
1	112	114	7.40	7.42
2	5,600	5,700	7.85	7.85
3	41	40	7.00	6.82
4	10,500	10,700	7.40	7.42
5	74	75	7.05	7.00
6	570	569	7.52	7.53
7	1,850	1,870	7.70	7.71
8	265	265	7.30	7.36
9	1,260	1,270	7.45	7.49
10	468	473	8.40	8.25
11	2,080	2,120	7.95	7.92
12	443	442	8.21	8.15
13	475	479	6.45	6.58
14	750	759	7.50	7.42
15	152	150	6.60	6.65

A study was made to determine if the sample temperature would effect the specific conductance. Standard solutions with specific conductances of 1,000 μmho cm^{-1} were cooled to temperatures ranging from 0°C to 18°C before they were introduced into the analytical system; no significant differences were noted. The specific conductance values ranged from 995 to 1,000 μmho cm^{-1} with no specific correlation with temperature. It was concluded that the thermostated heating coil and chamber has the capacity to equilibrate varying sample temperatures to 30°C under normal laboratory conditions.

Conclusions

This automated flow-through system has the ability to determine specific conductance and pH simultaneously with excellent accuracy and precision, and can accommodate the widely varying concentrations often encountered in natural water systems.

The authors thank Mr. Brian Millspaugh (DigiTec Corporation) for advice concerning the design of the digital readout and control devices, and the U.S. Geological Survey's Denver Central Laboratory for performing the manual specific conductance determinations.

TABLE 3

Carryover study. The samples were run in the order listed

Sample	Specific conductance (μmho cm^{-1})	Accepted value (μmho cm^{-1})
Demineralized water	2	—
6 X 1,000[a]	5,702	—
Sample 4[b]	101	96.9
6 X 1,000	5,696	—
Demineralized water	6	—
8 X 1,000	7,525	—
Sample 4	102	96.9
8 X 1,000	7,525	—
Demineralized water	8	—
10 X 1,000	9,264	—
Sample 4	106	96.9
10 X 1,000	9,269	—
Sample 2	315	307.4
15 X 1,000	13,357	—
Sample 2	321	307.4
15 X 1,000	13,352	—
1 X 1,000	1,014	1,000
4 X 1,000	3,860	—
Demineralized water	5	—

[a]Notation indicates that the KCl concentration is 6 times greater than a solution having a specific conductance of 1,000 μmho cm^{-1} (0.00702 M KCl).
[b]Samples 2 and 4 as listed in Table 1.

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THE DETERMINATION OF CHROMIUM SPECIES IN NATURAL WATERS

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(Received 19th January 1978)

SUMMARY

A method for the determination of chromium species has been developed and successfully applied to both fresh and sea water samples. The method utilizes pre-concentration of total chromium, chromium(III) and particulate chromium at natural pH with accurate and precise analysis by a single flameless atomic absorption procedure. A minimal blank allows for a reliable detection limit of 0.02 nM, which is sufficient for most natural waters with chromium concentrations in the range 0.01—10 nM. Immediate shipboard preconcentration of the samples minimizes storage problems. The method is simple and rapid; 20 samples can be analysed in duplicate for total chromium, chromium(III) and particulate chromium in one day with routinely available reagents and equipment.

Accurate and precise analyses of metal species are required in order to understand geochemical systems in natural waters. Total metal ion concentrations are not sufficient to predict the pathways of many elements. Chromium exists in natural waters in various forms, with different thermodynamic and kinetic characteristics [1] and toxicity effects [2]. Elderfield [3] suggested that chromium(III) and (VI) are the only significant oxidation states in natural waters, the most probable species being $Cr(OH)_2^+ \cdot 4H_2O$ and CrO_4^{2-}, respectively. In oxidizing waters, thermodynamic calculations predict that the stable form should be chromium(VI); however, significant amounts of chromium(III) have been found, as reviewed by Brewer [4]. The variety of techniques used to determine chromium species (Table 1) may account for some of the discrepancies in the oxidation state data [4].

The major difficulties that arise in studies of trace metal speciation in natural waters include the provision of sufficient precision and sensitivity to detect nanomolar concentrations; contamination during sampling; and species alteration during sample handling and analysis. Some methods are not applicable to the varied matrices of both fresh and sea water. Other methods may require analytical schemes that are time-consuming, may allow only a few samples to be determined daily, or may require specialized and expensive reagents and equipment dedicated to one element or species.

The aim of this project was to develop a sensitive, accurate and precise method to determine total chromium, chromium(III), and particulate

TABLE 1

Various methods for chromium determinations in natural waters

Pre-concentration method	Analytical technique	Detection limit[a] (nM)	Reference
None	D.p.p.	200 Cr(VI)	5
$Al(OH)_3$ co-pptn.	Flame a.a.s.	20 Cr(III)	6
None	N.a.a.	10 Cr (total)	7
Chelate resin	X.r.f.	5 Cr(III, VI)	8
Solvent extn.	G.l.c.	5 Cr(III)	9
Solvent extn.	G.l.c.	2 Cr (total)	10
Ion-exch. resin	Flame a.a.s.	2 Cr(III, VI)	11
Solvent extn.	Flame a.a.s.	1 Cr(VI)	12
None	Flameless a.a.s.	1 Cr (total)	13
None	Chemiluminescence	1 Cr(III)	14
$Fe(OH)_3$ co-pptn.	Colorimetric	0.4 Cr(III, VI)	4, 15
$Fe(OH)_3$ co-pptn.	Flameless a.a.s.	0.02 Cr(III, VI)	this work

[a]Many methods are designed for analysis of waste or polluted waters, thus their detection limits are not required to be low; with minor alterations, the detection limits may be improved in most cases.

chromium with existing a.a.s. equipment. Its application to shipboard work was also required so that sample storage problems could be minimized [12, 16, 17].

EXPERIMENTAL

Reagents

Stock solutions of chromium(III) and (VI) (0.01 M) were prepared by dissolving chromium(III) chloride and sodium dichromate in distilled, de-ionized water. Iron solutions (0.01 M) were prepared by dissolving iron(II) ammonium sulfate ("Baker Analysed" reagent) in distilled, de-ionized water. Hydrochloric acid was distilled in a quartz, sub-boiling still to remove impurities. Ammonium hydroxide (G. Frederick Smith ultraclean) was used to raise the pH of the iron co-precipitation solution.

Sample collection

Trace metal samples were collected in 5-l Niskin-type bottles, fitted with surgical rubber tubing closures, hung on stainless steel hydro wire, and tripped with brass messengers. Once removed from the wire, the bottles were placed in wooden storage racks; samples were drawn off through surgical tubing into 140-ml polyethylene bottles pre-cleaned with 1% hydrochloric acid at 60°C for 4 days. Care was taken not to introduce excessive air into the samples nor to leave an air gap prior to capping the bottle.

Co-precipitation

Chromium(III) is scavenged from a 140-ml sample by adding 1 ml of 0.01 M iron(III) hydroxide at pH 8 to the sample bottle and shaking for 1 h. Iron(III) solution is prepared by adjusting 0.01 M iron(II) ammonium sulfate solution to pH 8 with ammonium hydroxide and shaking for 1 day to oxidize the iron(II) hydroxide. The iron(III) hydroxide formed is stable as an effective scavenger for at least a month.

Both chromium(III) and (VI) are quantitatively co-precipitated by adjusting the iron(II) ammonium sulfate to pH 8 and immediately adding this green precipitate to a sample. The iron(II) hydroxide is prepared by adding 1 ml of 10% ammonium hydroxide to 50 ml of iron(II) ammonium sulfate. During shaking, the iron(II) hydroxide is oxidized and chromium(VI) is reduced to (III). Hem [18] suggested that at pH 8, the iron pair should maintain the pE of the solution at −3. As chromium reduction should occur at pE values below 6 [19], iron(II) is sufficient to reduce the chromium(VI). The iron(III) hydroxide scavenges the chromium(III), resulting in quantitative removal of all dissolved chromium. The iron(II) hydroxide has to be added to the sample within 10 min of its preparation.

After the samples have been shaken for at least 1 h, the solution and iron precipitate are vacuum-filtered through a 25-mm fritted glass system with 0.4-μm Nuclepore membrane precleaned with 12 M hydrochloric acid. The filters are handled with nylon forceps and stored in 50-ml flip-top polyethylene vials. Particulate chromium is collected by filtering a 140-ml sample with no iron hydroxide additions.

The co-precipitation step is conveniently carried out on board ship to minimize sample storage problems. Later, in the laboratory, the vials containing the filters and the concentrated chromium samples are treated with 1 ml of 6 M hydrochloric acid to dissolve the precipitate prior to analysis by flameless a.a.s.

Instrumental

A Perkin-Elmer Model 306 atomic absorption spectrophotometer with a Model 2100 Heated Graphite Atomizer was used under the instrumental conditions recommended by the manufacturer. The peak height range was established by varying the sample injection volume from 10 to 100 μl (Eppendorf pipettes) and by varying the recorder expansion scale from 1 to 10 mV. The optimum graphite atomizer conditions are: (a) dry for 35 s at 95°C for a 25-μl sample injection; (b) ash with a uniform temperature ramp to increase the temperature of the graphite tube from 95 to 1000°C over 20 s and hold at 1000°C for 5 s; (c) atomize at 2700°C for 5 s in a 50 ml min^{-1} flow of nitrogen.

Background correction for non-specific absorbance by cyanogen formed in the tube or salt particles is made with a deuterium arc corrector. For the chromium wavelength (357.9 nm), the effectiveness of the corrector is limited and this lowers the overall sensitivity; by comparing results for

various samples and standards with and without the corrector, it was concluded that the corrector is beneficial only when the graphite tube has deteriorated badly. The major salt that blocks the light path is iron(III) chloride which is removed during the ashing cycle as it vaporizes at 315°C.

Standards prepared from either chromium(III) chloride or sodium dichromate with appropriate acid and iron additions are used to produce a standard curve. It is mandatory to use the same injection volume for samples and standards. Working ranges can be changed by selecting a new injection volume or by dilution of the standards.

Calculations

Blank values for bottles, filters, vials and reagents are subtracted from absorption peak heights for total chromium, chromium(III) and particulate chromium. An overall blank concentration of 0.05 nM is routinely obtained. For anoxic waters of Saanich Inlet where thermodynamic calculations suggest that no chromium(VI) should be present, an average of 0.23 nM Cr(VI) was found. This could be interpreted as a maximum blank for the total chromium analysis.

Sample concentrations are obtained by interpolation from a least-squares calibration line. To determine the chromium(III) concentration, the sample preconcentrated on iron(III) hydroxide is corrected for particulate chromium and the blank. To determine the chromium(VI) concentration, the iron(II) hydroxide scavenged sample is corrected for chromium(III), particulate chromium and the blank.

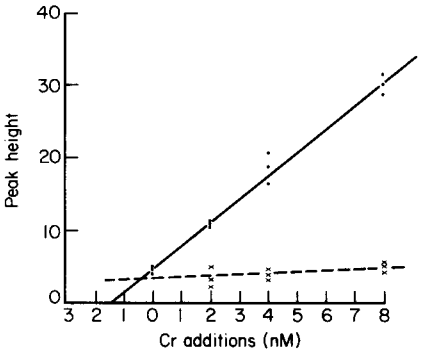
RESULTS AND DISCUSSION

Selectivity

Figure 1 contains results for standard additions to sea water samples, adding triplicate portions of chromium(III) and (VI) with iron(III) hydroxide as the scavenging agent. The chromium(III) additions were recovered while the chromium(VI) additions did not increase the absorption signal. As only chromium(III) is readily adsorbed by the iron(III) hydroxide, these results imply that chromium(III) can be recovered while chromium(VI) is not. When iron(II) hydroxide was added to triplicate standard additions of chromium(III) and (VI), both types of chromium were recovered (Fig. 2).

Accuracy, precision and detection limit

To determine the accuracy of the method, spike experiments were performed to test the recovery efficiency of each chromium species and iron hydroxide type. Table 2 contains the recovery data for 10 additions to surface sea-water samples (Puget Sound, Washington). Both chromium types were recovered by the iron(II) hydroxide additions. However, only chromium(III) additions were recovered by iron(III) hydroxide. The particulate matter appeared to collect some chromium(III) in cases where



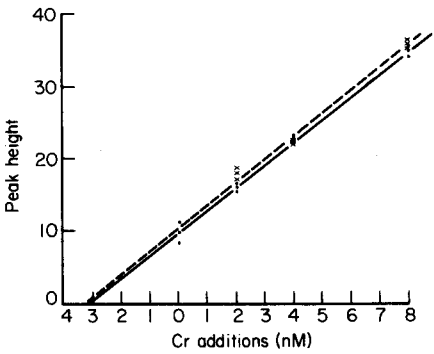


Fig. 1. Standard additions of Cr(III and VI) co-precipitated with $Fe(OH)_3$. (•) Cr(III) additions; (×) Cr(VI) additions.

Fig. 2. Standard additions of Cr(III and VI) co-precipitated with $Fe(OH)_2$. (•) Cr(III) additions; (×) Cr(VI) additions.

TABLE 2

Recovery of chromium spikes from surface sea water

Species sought	Fe type	Recovery efficiency (% ± 1 s.d.)	
		Cr(III) added	Cr(VI) added
Cr (total)	$Fe(OH)_2$	93 ± 8	91 ± 8
Cr(III)	$Fe(OH)_3$	83 ± 7	1 ± 2
Cr (particulate)	none	8 ± 11	0 ± 0

TABLE 3

Precision for triplicate samples

Cr type	Av. conc. (nM)	Mean s.d. for 11 sets of triplicates (± nM)
Cr (total)	2.60	0.12
Cr(III)	0.17	0.05
Cr (particulate)	0.12	0.05

the suspended load was large. Efficiencies of less than 100% probably result from adsorption on the container walls and filtration equipment.

Table 3 contains data for 11 sets of triplicates to indicate the overall precision of the method. The detection limit, defined as a signal/noise ratio of 2:1, was found to be 0.02 nM chromium in 140 ml of sample.

Contamination

Sampling systems were compared by means of 30-l Nisken bottles, special 5-l and 30-l gas-sampling bottles from Oregon State University and an in-situ pumping system. Variations larger than reagent blanks were not observed in any of the samples from the different samplers. Many of the chromium(III) and particulate chromium concentrations were equal to the blank concentration, indicating that contamination was not greater than the blank value.

In earlier work on iron hydroxide co-precipitation (e.g. ref. 15), a limiting factor was the high chromium background levels in the iron salts. Large sample volumes, or extensive purification of the iron source, were required to obtain satisfactory sensitivity. A study of many iron salts showed that iron(II) ammonium sulfate ("Baker Analysed" reagent) gave undetectable background chromium and allowed the use of small sample volumes and no purification steps. Also, use of the same iron source and instrumental procedure for both total chromium and chromium(III) analysis eliminates the problems caused when different blanks and sensitivities are used for different species.

Natural water concentrations

Table 4 shows some natural water chromium concentrations. Columbia River and Estuary water contain ca. 5 nM total chromium; chromium(VI) is the dominant form and more than 30% of the total is particulate chromium.

The north-east Pacific Ocean surface water (0—100 m) contains less total chromium than the deep water (all station depths greater than 2000 m.) Figure 3 contains chromium(III) and (VI) data for one location in the north-east Pacific. A small peak for chromium(III) at 75 m correlates with the primary nitrite maximum and may be related to biological activity; the increase in chromium(VI) with depth correlates strongly with dissolved silica, suggesting that a portion of the chromium may be involved in a deep regeneration cycle [20].

TABLE 4

Determination of chromium species in some natural waters (in nM)

Environment	*n*	Cr(VI)	Cr(III)	Cr(partic.)	Cr(total)
Columbia River	3	3.3 ± 0.6	0.09 ± 0.13	1.8 ± 0.1	4.9 ± 0.5
Columbia R. estuary	10	2.4 ± 0.7	0.32 ± 0.38	2.3 ± 1.3	4.7 ± 1.1
N.E. Pacific Ocean surface (0—100 m)	49	2.0 ± 0.4	0.15 ± 0.13	0.05 ± 0.05	2.4 ± 0.7
N.E. Pacific Ocean deep (below 100 m)	40	2.8 ± 0.7	0.04 ± 0.04	0.05 ± 0.04	3.1 ± 0.5
Saanich Inlet, B.C. oxygenated waters	11	1.5 ± 0.3	0.08 ± 0.08	0.14 ± 0.10	1.7 ± 0.3
Saanich Inlet, B.C. anoxic waters	8	0.23 ± 0.14	0.84 ± 0.15	0.33 ± 0.12	1.4 ± 0.1

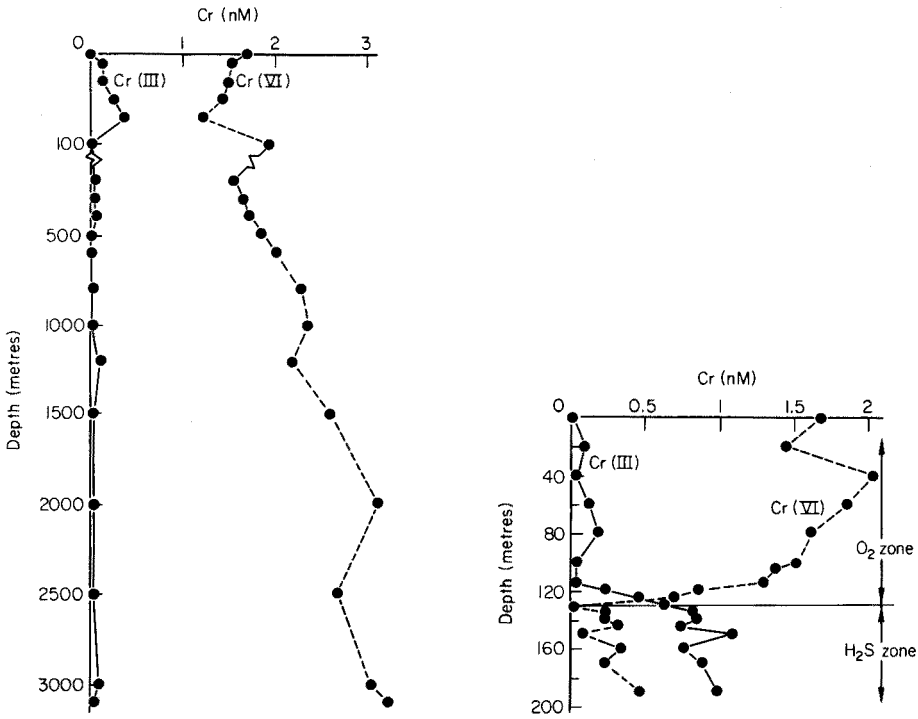


Fig. 3. Chromium species in N.E. Pacific Ocean waters (July, 1977; 132° 50.6W, 47° 01.5N).

Fig. 4. Chromium species in Saanich Inlet, B.C., Canada (July, 1977; 123° 32.7W, 48° 33.1N).

In oxygenated fjord samples from Saanich Inlet, chromium(VI) is the dominant species; the anoxic zone with H_2S present contains chromium(III) as the major species (Fig. 4). The high particulate chromium concentrations in the anoxic water (Table 4) indicate that chromium(III) may be adsorbed onto suspended matter and removed from the water column by settling.

These data agree with thermodynamic prediction that chromium(VI) is the dominant species in oxygenated water, with variations occurring for chromium(III) and particulate chromium related to biological and/or other natural processes.

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IRRADIATION TECHNIQUES FOR THE RELEASE OF BOUND HEAVY METALS IN NATURAL WATERS AND BLOOD

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SUMMARY

Irradiation techniques are compared with conventional acid digestion procedures for the release of bound heavy metals in natural waters and in blood, before their determination by anodic stripping voltammetry. Ultra-violet irradiation of acidified water with a 550-W mercury vapour lamp releases bound zinc, cadmium, lead and copper after 4 h. The same results can be achieved with a 30-Mrad dose of high-energy γ-irradiation. These techniques are also effective for the release of metals in whole blood and blood plasma, where sample volumes as small as 200 μl are adequate in analyses for zinc, copper and lead. By comparison with acid digestion and solvent extraction methods, irradiation treatments offer the advantages of minimum sample manipulation and negligible reagent blanks.

The direct determination of trace concentrations of heavy metals in environmental water samples or in blood may be complicated by the presence of matrix components which either suppress or mask the analytical signal. Natural waters have a dissolved organic carbon (DOC) content in the range 1—30 mg C l^{-1} and a dissolved salt component as high as 580 meq l^{-1} in sea water. Whole blood typically contains greater than 80 g C l^{-1} and 100 meq l^{-1} of dissolved salts, while in blood plasma the values are 260 mg C l^{-1} and 130 meq l^{-1}, respectively.

Atomic absorption spectrophotometry (a.a.s.) is now widely used for the analysis of natural waters for trace metals. With graphite furnace atomization, high sensitivity is attainable for most metals of interest in fresh waters; however, the analysis of saline waters is complicated by light scattering and background molecular absorption. Even though these may be accounted for instrumentally, sensitivities are often insufficient to detect the natural concentrations of some metals. The analysis of blood for trace metals by a.a.s. has been investigated extensively [1]. For toxic metals such as lead and cadmium, present in whole blood at μg l^{-1} concentrations, a preliminary acid digestion [2] and/or solvent extraction [3] is generally incorporated in the analytical procedure.

Anodic stripping voltammetry (a.s.v.) has found increasing acceptance for the measurement of trace levels of heavy metals. For those metals to which

it can be applied, a.s.v. offers considerably greater sensitivity than a.a.s. However, unlike a.a.s. the technique does not measure total metal concentration, but only the labile metal fraction, i.e. that which can be dissociated under the electrolysis conditions. Solution pretreatments must be employed before the measurement step, to release bound metal from inorganic or organic complexes or metal which is adsorbed on colloidal particles. A potential matrix interference can arise from less than millimolar concentrations of certain surface-active organic compounds, which can lead to depression of stripping peaks as a result of film formation on the electrode preventing metal deposition. This is frequently observed with polluted industrial waste waters or with organic-rich fresh waters and can lead to erroneous results.

Heavy metals present in blood will be complexed, if only weakly, by the naturally present organic species such as sugars, sterols, fats and proteins. It is well known, for example, that more than 90% of the manganese and lead in blood is weakly bonded to red cell proteins [1]. Release of this metal requires either decomposition of the complex [4] or exchange with a more strongly complexed metal such as chromium(III) [5].

The commonest procedure for the destruction of organic matter in trace metal analysis is a wet oxidation, generally with nitric and perchloric acids. Acid decomposition has been applied to analysis by a.s.v. of both blood [4] and natural waters [6]. As will be shown later, this procedure is liable to contamination from the added reagents and may introduce metals at concentrations which exceed those present naturally. For sea water, which is generally low in dissolved organics (less than 5 mg C l^{-1}), Batley and Florence [7] have shown that boiling with dilute nitric acid is sufficient to release bound metals. For fresh waters, however, total acid digestion of the sample is necessary [6]. Various possible pretreatment methods for the release of total metals have been summarized by Fukai and Huynh-Ngoc [8]. These include persulfate oxidation and APDC—MIBK extraction. Apart from impurity problems, the value of persulfate has been questioned because of the incompleteness of the oxidation [9]. Similarly, chelation—extraction methods may not necessarily remove all metal species [10].

In 1966, Armstrong et al. [11, 12] demonstrated that organic matter in sea water could be easily decomposed by u.v. irradiation. Using a medium-power mercury arc lamp, they showed that irradiation for up to 12 h was sufficient to oxidize 98% of the dissolved organic carbon, nitrogen and phosphorus compounds in sea water. Manny et al. [13] reported similar data for lake waters. This technique was subsequently used in studies of metal speciation to release metals associated with organic compounds in natural waters [9, 14—16]. Used on unacidified samples, the technique will not yield total metal concentrations, and the large metal fraction associated with inorganic colloidal species or strong inorganic complexes will remain undissociated during a.s.v. measurements [7]. This fraction is released on warming the sample with dilute acid.

High-energy ionizing radiations such as γ-rays can also be used to destroy organic matter [17]. In aqueous solution the absorption of γ-radiation will result in the formation of molecular hydrogen and hydrogen peroxide, together with hydrogen atoms ($H^{\cdot}$), solvated electrons (e^{-}_{aq}), and hydroxyl ($OH^{\cdot}$) and perhydroxyl ($HO_2^{\cdot}$) radicals [17, 18]. The nature and rate of formation of the primary radiation products will be pH-dependent. In the acidic conditions required for metal release from colloidal species, and in the presence of dissolved oxygen and hydrogen peroxide, the release of heavy metals from organic complexes will be brought about by oxidation and radical-initiated decomposition of the organic molecules by $OH^{\cdot}$, $HO_2^{\cdot}$ and H_2O_2 .

This paper deals with the use of acids, ozone, low-energy u.v. irradiation and high-energy γ-irradiation for the decomposition of organic matter and the release of bound metals from difficult matrices such as natural waters and blood.

EXPERIMENTAL

Apparatus

Anodic stripping voltammetric measurements of zinc, cadmium, lead and copper concentrations were made at a hanging mercury drop electrode with a PAR 174 polarographic analyser in the differential pulse mode as described previously [7].

Ultra-violet irradiations were done with a 550-W mercury arc lamp in a rig similar to that described by Armstrong and Tibbitts [12]. Samples were contained in 23 × 3.2-cm silica test tubes and covered with watch glasses. Also tested was a 36-W immersible U-tube lamp (Oliphant Pty. Ltd., Sydney) which was immersed in a stirred sample contained in a 1-l measuring cylinder. A Blak-ray u.v. meter model J-225 (U.V. Products Inc., U.S.A.) was used to measure u.v. radiation flux.

The fuel element storage facility at the Australian Atomic Energy Commission Research Establishment (AAECRE) was used for γ-irradiations. A dose rate of 560 rad s^{-1} was used. Samples were contained in 50-ml borosilicate glass tubes having screw caps with Teflon liners. Tubes were thoroughly rinsed with acid and pre-irradiated before use.

Measurements of organic carbon were performed on an Oceanographics Carbon Analyser.

Reagents

Nitric acid, perchloric acid, sodium chloride and sodium acetate were Merck 'Suprapur' grade; hydrogen peroxide was A.R. grade.

Distilled water was prepared from deionized water by distillation in the presence of potassium permanganate in an all-glass still.

A lead nitrate-dosed freeze dried blood (Tox-el (Pb)) was supplied by A. R. Smith Laboratories, Calif., U.S.A. Whole blood and plasma were obtained from donors at the AAECRE, Lucas Heights.

U.v. Irradiation

Transfer the water sample (100 ml), previously filtered through a 0.45-μm membrane filter, to the silica irradiation tube. Add 0.70 ml of concentrated nitric acid and 0.05 ml of 30% hydrogen peroxide and cover with a watch-glass. Irradiate the sample for 4 h, with a 550-W lamp. Cool and transfer to the a.s.v. cell.

For blood analyses, add 0.200 g of whole blood, 0.100 g of freeze-dried blood or 0.50 ml of blood plasma to 100 ml of 0.5 M sodium chloride containing nitric acid and hydrogen peroxide as before, and irradiate the mixture for 4 h prior to a.s.v.

γ-Irradiation

Add 25-ml aliquots of the above mixtures to capped borosilicate tubes. Expose to a total radiation dose of 30 Mrad from a γ-source, cool and then transfer to the a.s.v. cell.

Analyses for total metals

The procedures for total acid digestion and a.s.v. determination of heavy metals in waters were as described previously [7].

For acid digestion of blood samples, take 1 ml of whole blood or plasma or 1 g of freeze-dried blood in a 50-ml conical flask. Add 1 ml of concentrated nitric acid and boil gently on a hot plate. Add a further 0.5 ml of this acid and 0.7 ml of 70% perchloric acid and evaporate the solution to fumes of perchloric acid. Transfer to a 25-ml volumetric flask and dilute to volume with distilled water. Stripping analysis of the digest follows the method for waters [7].

RESULTS AND DISCUSSION

Analysis of natural waters

Samples of fresh water, sea water and water from a polluted stormwater canal, were analysed for total zinc, cadmium, lead and copper, and the results obtained by differing pretreatments for the release of bound metals were compared (Table 1). The methods comprised boiling with dilute acid, acid digestion, ozonolysis, u.v. irradiation and γ-irradiation.

Boiling with dilute nitric acid is effective in releasing bound metals in unpolluted sea water [7]. For sea-water samples containing high concentrations of organic pollutants, and for many fresh-water samples, a total oxidation treatment with nitric and perchloric acids is required to liberate all metal. Table 2 shows that reagent blanks for the acid digestion procedure are high, even when ultrapure reagents are used. These figures arise both from added acids, the least pure of which is perchloric acid, and from the added distilled water. It was also noted that random sample contamination could occur during evaporation of the samples on a hot plate even though the beakers were covered. This was particularly so when multiple analyses

TABLE 1

Release of bound metals from water samples

Sample	Treatment	Zn	Cd	Pb	Cu
		($\mu g\ l^{-1}$)			
Sea water, Cronulla, NSW	Boiling with 0.16 M HNO_3	1.0	0.31	0.50	0.54
	Acid digestion $HNO_3/HClO_4$	0.9	0.38	0.51	0.46
	U.v. irradiation	0.9	0.41	0.54	0.55
	γ-Irradiation	1.1	0.35	0.61	0.48
	Ozonolysis	0.47	0.25	0.40	0.38
River water, Magela Creek, NT	Acid digestion $HNO_3/HClO_4$	9.6	0.8	3.4	2.3
	U.v. irradiation	9.2	0.6	4.0	2.1
	γ-Irradiation	9.0	0.7	3.8	2.3
Alexandra Canal, Sydney, NSW	Acid digestion $HNO_3/HClO_4$	350	0.8	8.0	25
	U.v. irradiation	331	1.0	7.5	23
	γ-Irradiation	344	0.9	7.8	24

TABLE 2

Reagent blanks for a.s.v. measurements[a]

	Zn	Cd	Pb	Cu
	($\mu g\ l^{-1}$ in original sample)			
0.16 M HNO_3	0.08[b]	<0.001	0.001	0.001
0.16 M HNO_3/0.12 M $HClO_4$	0.75[b]	0.05	0.14	0.10
0.01 M H_2O_2/0.10 M HNO_3	0.03[b]	<0.001	0.001	0.001

[a]Merck Suprapur reagents.\[b]Requires addition of sodium acetate to pH 4.6.

were done on an aluminium heating block, which necessitated lengthy evaporation times if bumping of the sample was to be avoided.

Samples after evaporation to perchloric acid fumes had to be diluted with distilled water, blanks for which appeared sometimes to be greater than the original sample. For the a.s.v. of zinc, addition of sodium acetate was necessary to bring the pH to a value where the zinc wave was measurable, and this can also contribute a sizeable blank. By contrast, the acid and peroxide additions for the irradiation procedures contributed negligible blanks (Table 2). Sodium acetate additions for zinc determinations were also lower because of the lower acidity in the sample.

For u.v. irradiations, the two rigs described above were examined. With the 550-W mercury arc lamp, the solution temperature after 4 h had risen to 90°C. When a silica sheath was placed round the lamp, the measured incident radiation on the sample decreased from only 4.8 to 4.0 mW cm^{-2}, but the solution temperature did not exceed 50°C. The radiation dose received from this lamp by a 100-ml sample was compared with that from the 36-W immersion lamp by measuring the photolysis of 0.06 M potassium

ferrioxalate in 0.1 M H_2SO_4. Because of the rig geometry, the dose from the 36-W lamp was about 50% of the dose from the 550-W lamp. The solution temperature reached a maximum of 50°C. Although this lamp has the advantages of lower power requirements, it has the inconvenience that only one sample can be irradiated at a time, and both lamp and sample container need careful cleaning between samples.

The decrease in dissolved organic carbon was compared with the release of trace metals as a function of irradiation time for the three water samples. Samples were acidified to 0.1 M hydrogen ion and hydrogen peroxide was used as the source of oxygen free radicals. For sea water, DOC figures after irradiation for 1 h had diminished to less than 1 mg l^{-1} and could not be accurately measured with the available instrumentation. Measurement of the u.v. absorbance of sea water at 254 nm showed a decrease in absorbance of 85% after irradiation for 1 h and 90% after 2 h, confirming a rapid decomposition. Analyses for metals gave constant results after irradiation for 2—3 h (Table 3).

Armstrong and Tibbitts [12] observed higher rates of oxidation for sea water than fresh water. For the river-water sample (Fig. 1), however, metal release was also essentially complete after 2—3 h, although destruction of organic matter was incomplete, with 20% remaining unoxidized. This is not unexpected because the oxidation of molecular side chains containing metal-binding groups is likely to precede oxidation of the benzene rings which represent the major carbon content of molecules such as humic acids.

Allen [19] examined the u.v. photo-oxidation of molecular size fractions of organic matter in a soft lake water. Approximately one third of the DOC content was associated with the >10,000 molecular weight and a similar amount with the <500 fraction. These two size fractions consistently showed the most rapid and complete oxidation, while intermediate molecular weights were refractory to u.v. decomposition. Allen noted that maximum "chelation" occurred with the fractions of highest molecular weight; this study is supported by the results of Giesy and Briese [20] for swamp and river waters and by Smith [21] for estuarine waters.

TABLE 3

Release of metals from sea water by u.v. irradiation

Time (min)	Cd	Pb ($\mu g\ l^{-1}$)	Cu
0	0.18	0.31	0.30
45	0.24	0.38	0.35
90	0.30	0.37	0.37
120	0.36	0.53	0.37
150	0.35	0.55	0.47
240	0.41	0.55	0.51
360	0.38	0.54	0 55

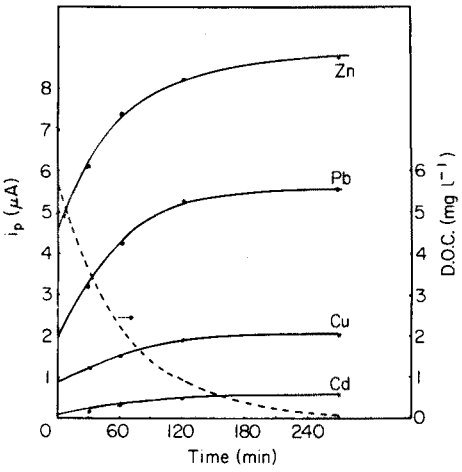

Fig. 1. Effect of time of u.v. irradiation on labile Zn, Cd, Pb and Cu, and on dissolved organic carbon for a river-water sample.

The Alexandra Canal (NSW) sample had a high initial DOC of 28 mg C l^{-1} and a high labile metal concentration. Anodic stripping voltammograms on the untreated sample were distorted, and peaks were suppressed by the presence of adsorbed organics. Measurements of labile metal vs. irradiation time for acidified samples showed constant results after 2 h (Table 4).

To cover the range of dissolved organics likely to be encountered in natural waters, an irradiation time of 4 h was selected as desirable for metal release. This method has been used routinely over the past year. Compared with the acid digestion procedure, detection limits are lower, precision is greater, and random errors are absent.

The use of high-energy γ-irradiation was investigated as an alternative to low-energy u.v. irradiation as a technique for the release of bound metals. Optimum conditions for metal release were assessed from plots of measured labile metal concentrations and DOC as functions of radiation dose. The

TABLE 4

Release of metals from Alexandra Canal water using u.v. irradiation

Time (min)	DOC (mg l^{-1})	Zn	Cd	Pb	Cu
			(μg l^{-1})		
0	8.2	—[a]	—[a]	—[a]	—[a]
30	4.6	300	0.9	7.3	15
60	0.8	330	0.8	7.7	17
120	0.5	335	1.0	7.5	24
240	0.2	331	1.0	7.5	23

[a]Stripping peaks depressed by adsorbed organics.

addition of hydrogen peroxide was essential for formation of oxidizing radicals. Typical results for a river water (Table 5) led to the selection of 25—30 Mrad as an adequate dose to effect complete metal release, although complete decomposition of organic matter was not achieved. Results obtained by this method compare well with those obtained after u.v. irradiation (Table 1). The sample temperature increased to an estimated 60°C during irradiation. The purity of the irradiation tubes with respect to trace metals was equally important in γ-irradiation experiments. In addition to hot acid leaching, the Pyrex glass tubes, containing dilute acid blanks, were irradiated before use for water samples.

The technique offers considerable advantages if an irradiation facility is available. Large numbers of samples may be handled simultaneously, and sample containers are inexpensive (Pyrex glass vs. silica). A more detailed study is being made of the use of γ-irradiation for the determination of organic nitrogen, phosphorus and carbon in sea-water samples.

Clem has alluded to the use of ozone as an oxidant for the release of bound metals [22]. However, when ozone treatments were examined here, in both neutral and acidified solutions, with and without heating, no significant metal release was achieved, even after treatment with ozone for 30 min.

Analysis of blood

Samples of whole blood, freeze-dried blood and blood plasma were subjected to acid digestion, u.v. irradiation and γ-irradiation to release bound metals prior to a.s.v. (Table 6). The high organic content of whole blood severely restricted the sample size that could be used. For acid digestions, sample volumes of 1—5 ml were manageable but required careful heating to avoid frothing and loss of sample. Complete destruction of organics by irradiation methods was not achieved if the whole blood concentrations exceeded 0.5 ml in 100 ml of 0.5 M NaCl. Partially decomposed cell protein agglomerated in the irradiation tube and required filtration before analysis, while decomposition intermediates frothed on deaeration prior to a.s.v. and gave rise to interfering peaks. This could be overcome by the use of smaller samples. This limit did not apply to plasma samples for which up to 1.0 ml gave no interferences.

The whole blood used was weighed directly and hemolysed with water immediately after collection, although no problems were encountered with

TABLE 5

Effect of γ-irradiation dose on dissolved organic carbon content of river water

Radiation dose (Mrad)	0	0.5	2.0	5	10	20	30
DOC (mg l^{-1})	25	18	8	4.5	2.8	2.0	1.8
Cu ($\mu g\ l^{-1}$)	0.5	1.0	0.9	1.5	2.2	2.1	2.2

TABLE 6

Determination of Pb and Cu in blood

Sample	Treatment	Dose	Pb ($\mu g\ l^{-1}$)	Cu ($\mu g\ l^{-1}$)
Whole blood	$HNO_3/HClO_4$	—	0.14	1.2
	U.v. irradiation	6 h	0.14	1.0
	γ-Irradiation	30 Mrad	0.13	1.2
Freeze-dried[a] blood	$HNO_3/HClO_4$		0.63	1.0
	U.v. irradiation	4 h	0.61	1.1
	U.v. irradiation	6 h	0.69	1.0
	γ-Irradiation	10 Mrad	0.50	1.2
	γ-Irradiation	20 Mrad	0.62	1.0
	γ-Irradiation	50 Mrad	0.60	1.0
	γ-Irradiation	130 Mrad	0.62	1.0
Blood plasma	$HNO_3/HClO_4$	—	0.06	1.6
	U.v. irradiation	6 h	0.07	1.8
	γ-Irradiation	50 Mrad	0.06	1.8

[a]Pb = 0.63 ± 0.07 $\mu g\ l^{-1}$ from interlaboratory survey.

stored samples to which the anticoagulants heparin or EDTA had been added, since both compounds can be decomposed by irradiation. Irradiation was carried out in dilute acid and in acidified dilute NaCl; the latter was preferred because it provided a suitable base electrolyte for a.s.v.

For whole blood samples from healthy donors, the analytical signals obtained from 0.2-g samples were greater than 10 times the detection limit for lead, and 100 times the limit for copper and zinc. Normal levels of $2\ \mu g\ l^{-1}$ reported for cadmium in whole blood [4] were not detectable with this size of sample, but cadmium concentrations considered worthy of further clinical investigation ($50\ \mu g\ l^{-1}$ [1]) would still give a measurable signal.

Results obtained by irradiation techniques were comparable with those by acid digestion treatments. Required irradiation doses were 4—6 h for u.v. irradiation and 20—30 Mrad for γ-irradiation.

The freeze-dried blood used in these studies was a lead nitrate-doped sample used in an international interlaboratory survey of lead in blood analysis conducted jointly by the University of NSW and The Royal Australian Chemical Institute. Good agreement was found with the mean value obtained from this survey (Table 6).

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DETERMINATION OF TOTAL MERCURY IN SEDIMENTS BY FURNACE COMBUSTION AND PLASMA EMISSION SPECTROMETRY

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SUMMARY

A technique for estimating nanogram quantities of mercury in sediment samples is described. Samples are heated at 870°C in an oxygen atmosphere, and the released mercury is collected on a gold-coated glass bead trap. The trap is then heated to 500°C in a helium stream; mercury is swept through a d.c. discharge cell and measured by emission spectrometry. Recoveries of mercury added to sediment samples were quantitative. Comparison of five separate samples by the proposed method and by a conventional cold-vapor atomic absorption technique showed similar results. The proposed method gives linear calibration plots up to 1700 ng Hg; the detection limit is 10 ng Hg, so that the sensitivity is 0.005 ppm for a 2.0-g sample.

Increased concern over the toxic potential of mercury in the environment has stimulated a search for analytical techniques that are rapid, simple and sensitive. Reimers and Krenkel in an extensive review [1], have summarized existing methods for total mercury determinations in environmental samples. Most methods require tedious acid digestion periods for the destruction of organic material, or several days of elapsed time as in neutron activation techniques, hence the development of rapid combustion techniques is desirable.

The method described here involves the combustion of sediment samples in oxygen to destroy potentially interfering organic material and collection of the released mercury by amalgamation on a gold-coated glass bead trap. The mercury is then determined by emission spectrometry. The method is relatively rapid, requiring less than 45 min per analysis, involves no reagent addition and minimizes the chance of contamination because of its simplicity. With a system blank of less than 6 ng Hg, the detection limit is 0.005 μg Hg g^{-1} of sediment for a 2.0-g sample.

Amalgamation of mercury on gold is utilized for cleansing the oxygen flow before its entry into the combustion tube, and for the quantitative collection of the mercury released from the sediments on heating.

EXPERIMENTAL

Apparatus

The combustion and absorption apparatus are shown in Fig. 1. An 870°C electric tube furnace (32.5-cm long with a 3.6-cm diameter insertion port) was utilized. Samples were placed in a quartz tube, 75 cm long, with ground-glass joints for fitting the gold bead traps. A "sediment boat" — a 7 × 1-cm quartz semi-cylinder — was used for loading samples which were inserted into the quartz combustion tube by a glass rod.

For the final measurement, the mercury emission line was isolated with a monochromator and the emission intensity was measured with a conventional photomultiplier and chart recorder [3].

The quartz sediment boats and the glass rod used to insert the boat were washed with hot soapy water before each analysis. The quartz combustion tube was heated in the furnace in a mercury-free oxygen flow (described below) for 10 min prior to each analysis. System blanks were obtained periodically and were consistently less than 6 ng Hg.

The procedure for gold-plating glass beads has been described and assessed by Braman and Johnson [2].

Calibration of instrument

A standard curve was established daily. This was conveniently done by withdrawing a definite volume of mercury-saturated air with a 2.5 ml syringe from a sealed test tube immersed in a water bath of known temperature, containing a drop of pure mercury. The mercury vapor was deposited on a gold bead trap by slowly injecting the withdrawn air into the top of the quartz tube while applying a negative pressure (by vacuum) at the bottom of the tube. The trap was then analyzed by emission spectrometry [2]. The amount of mercury added was calculated from temperature and vapor pressure data and related to peak area in the recorded response. As illustrated in Fig. 2, the gold bead trapping system and emission analysis gives linear responses in the ranges 10—1700 ng Hg. 10—90 ng Hg (correlation coefficient, 0.980) and 150—1700 ng Hg (correlation coefficient, 0.983).

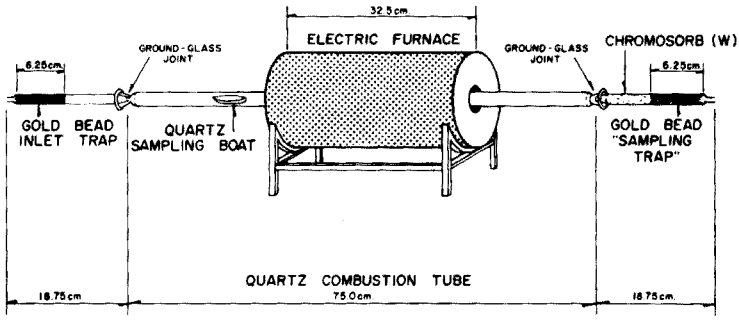

Fig. 1. Combustion and absorption system.

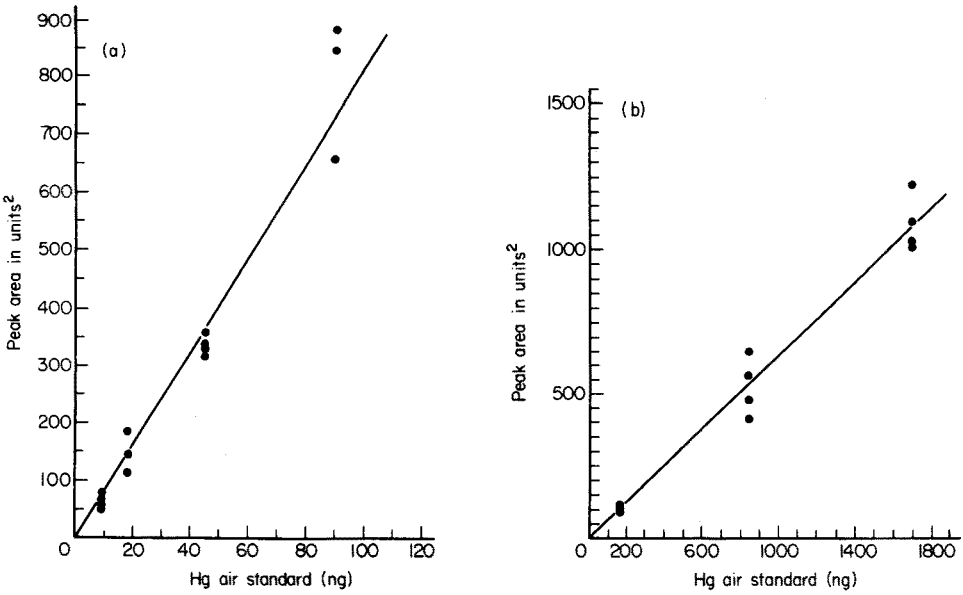

Fig. 2. Calibration graphs for mercury air standard. (a) 9.0—90 ng Hg; slit width, 60 μm; PM voltage, 360 V. (b) 150—1700 ng Hg; slit width, 40 μm; PM voltage, 300 V.

Procedure

The sediment sample (0.25—2 g) was loaded onto a clean quartz sampling boat, which was placed into the quartz combustion tube, and pushed in with a clean glass rod to a position just adjacent to the furnace (Fig. 1). The gold bead "inlet trap" was then connected to the combustion tube through which was passed the oxygen carrier gas. The gold bead "sampling trap", containing Chromosorb W as shown in Fig. 1, was also connected up. This sampling trap was purified previously, by heating, in a helium carrier gas, the area containing Chromosorb W with a nichrome heating coil at ca. 250°C, and the area containing the gold bead packing at 500°C.

The oxygen flow rate was adjusted to 200 ml min^{-1} and the sample area of the combustion tube was slowly inserted into the furnace. After 2—3 min, when most of the moisture had been driven from the sample, the sample area was pushed into the hottest part of the furnace, the oxygen flow rate was increased to 1 l min^{-1}, and the combustion of sediments was allowed to proceed for 5—7 min. Any part of the combustion tube between the sample and the sampling trap was also placed inside the furnace for 2—3 min.

Sediment samples which are high in moisture and/or organic content will interfere with the emission process in the final step. A transfer step is one way of removing these interferences and was used in this study in the analysis of Onondaga Lake sediment samples.

After combustion, the sampling trap was removed from the combustion tube, and with a clean glass rod, the Chromosorb W was removed from the

trap. The trap was then connected to a gold bead "analysis trap", previously purified at 500°C, as illustrated by Fig. 3. Heat (about 800°C) was then applied to the sampling trap for 60 s in an oxygen flow of 1 l min^{-1}. After the heat had been turned off, the oxygen flow was increased to 3 l min^{-1} for another 30 s; double trapping experiments showed no leakage of mercury onto the second trap for this step at this flow rate. The analysis trap was then analyzed for total mercury by emission spectrometry [2].

Finally, the baked sediment was carefully weighed for dry-weight determination.

RESULTS AND DISCUSSION

To determine the efficiency of recovery of the entire combustion procedure, 392 ng and 981 ng of mercury were applied to sediments from a remote area (Lake Jimmy; Adirondack Park, N.Y.) which were found to be low in mercury by this method. The samples were then analyzed as described above. A mercury standard (as $HgCl_2$) was applied to the crushed and mixed samples with a 10-μl syringe just prior to the analysis. The results (Table 1) showed that the release of mercury was essentially quantitative.

However, sediment samples that were high in organic content (such as those from Onondaga Lake, N.Y.) caused a tailing effect in the recorded response because of interfering organic compounds. Therefore, two optional steps were added to the procedure for sediment samples of this type. First, the Chromosorb W packing in the sampling trap reduced the interfering effects, as evidenced by sharp distinct peaks in the recorded response at the 253.58-nm line. The Chromosorb W provided a high surface area for the adsorption of potentially interfering organic compounds. Secondly, the transfer step, described above, may convert any residual organic materials to carbon dioxide, and has the effect of driving off the moisture collected in the sampling trap. Reducing the oxygen flow rate during combustion may reduce the interference and moisture problems without the use of these steps.

Table 2 shows a comparison of the results obtained by the proposed method

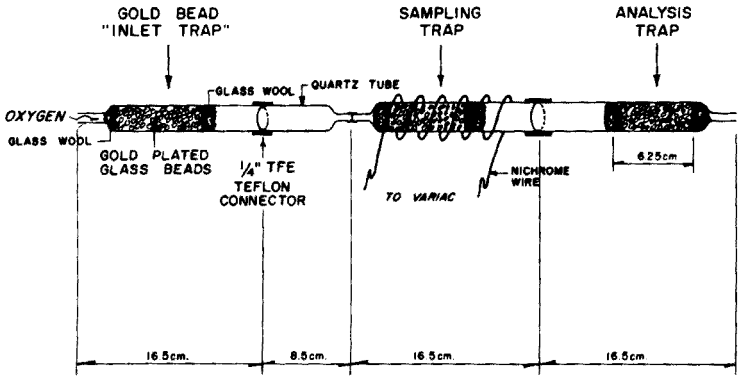

Fig. 3. Transfer to "analysis" trap.

TABLE 1

Recovery of added mercury from a sediment by the furnace technique (In each case — including the background determination — approximately 1.0 g (dry wt.) of sediment was used.)

Hg added (ng)	Hg recovered (ng)	Hg found after background correction (ng)	Recovery (%)
981	1108.5	846.1	86.2
392	667.3	404.9	103.3

TABLE 2

Comparison of the furnace and cold-vapor atomic absorption techniques

Sample	Atomic absorption average[a] ($\mu g\ g^{-1}$)	Furnace technique average[a] ($\mu g\ g^{-1}$)	Ratio Furnace/a.a.s. (%)
9	5.3	4.5	85.2
10	4.2	4.5	107.1
11	4.5	3.6	80
12	3.2	3.2	100
13	2.4	1.8	75

[a]Averages of triplicate analyses.

and by cold-vapor atomic absorption technique after acid digestion [5], for 5 samples taken from Onondaga Lake. These sediments are very fine grained, consisting of calcite and other carbonaceous material. The sediments have been found to be 10% organic matter by weight [4]. The results indicate that, in most cases, the furnace combustion method gives slightly lower values than atomic absorption analysis. The values, however, are close enough to verify the validity of the furnace combustion technique for routine analysis of sediment samples.

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GEL ELUTION OF HETEROCYCLIC ANALOGUES OF POLYNUCLEAR AROMATIC HYDROCARBONS FROM BIO-BEADS

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SUMMARY

The chromatographic elution characteristics from Bio-Beads SX-12 gel are reported for a wide range of polynuclear aromatic hydrocarbons and some of the heterocyclic analogues with which they are frequently associated in the environment. The use of benzene as solvent allows absorption effects to take place on the gels in the absence of hydrogen-bonding effects. The elutions observed are discussed in terms of the contribution of the heteroatom to the gel elution effects.

Polynuclear aromatic hydrocarbon (PAH) sources in the environment almost always also contain heterocyclic analogues of the PAH (X—PAH; X = N, O, S). Cigarette smoke condensate (CSC) contains N—PAH (indoles, carbazoles, and aza-arenes) and O—PAH (benzofurans, dibenzofurans, and benzonaphthofurans) in amounts equal to or greater than the PAH [1—4]. Aza-arenes occur in air pollution [5, 6] and, along with S—PAH (benzothiophenes), are abundant in petroleum fractions [7, 8] and coal-derived fuels [9]. Recent work has demonstrated the general utility of gel chromatography for isolating the polynuclear organic material (POM; including PAH and X—PAH) from these and other environmentally important sources [3, 4, 10—18]. Most of this work has involved Sephadex LH-20 (a partially alkylated cross-linked dextran) [19—25] and the Bio-Beads SX-series of gels (a styrene—divinylbenzene copolymer) [11, 12, 24—29]. With these gels, aromatic compounds are separated from aliphatic compounds by an adsorption-type mechanism. Further, when adsorption occurs, individual aromatic compounds are eluted from the gels in order of increasing ring number. This adsorption property has been extensively investigated for POM on LH-20 and PAH on Bio-Beads. Apparently, the order of PAH elution from these gels is solvent-dependent. In tetrahydrofuran [11, 22] and dimethylamine [25] both gels cause the larger PAH to elute first, as expected for gel permeation chromatography. LH-20 in dimethylformamide produces a similar effect [22]. Adsorption of PAH occurs when LH-20 is eluted with methanol [21—24], isopropanol [19—24], acetone [24], and acetonitrile [22, 24], and when Bio-Beads are eluted with acetone [24, 26],

methyl ethyl ketone [24], methylene chloride [28], and benzene [3, 4, 18, 27, 29]. No adsorption or permeation of PAH is observed with Bio-Beads in methanol, isopropanol [19—24], or acetonitrile [30]. The adsorption effect of PAH appears to be due to a π—π-type interaction between the PAH and the gels (LH-20 and Bio-Beads) and has been correlated with the resonance energy of the PAH (LH-20 system) [21, 23].

With the extensive use of gel chromatography for isolation of POM and the almost universal co-occurrence of PAH and X—PAH, the possibility of the presence of X—PAH in the isolated fraction must be considered. It thus becomes important to determine the elution characteristics of X—PAH in order to utilize fully the separation properties of the gels. Few reports have been published on the elution of heterocyclic analogues of PAH from LH-20 or Bio-Beads. Klimisch and Reese [11] found that carbazoles and acridines were not adsorbed by Bio-Beads in THF; the larger molecules were eluted first as with PAH. Streuli [21—23] investigated the elution of a number of heterocyclics from LH-20 in various solvents. Although he obtained a good correlation of elution with resonance energies [21] or delocalization energies [23] for PAH, the relationship did not hold for heterocyclics. Streuli attributed the differences observed to hydrogen bonding between the solute and the LH-20 gel [23]. To our knowledge there are no reports in the literature on the gel filtration chromatography of heteroatom PAH with Bio-Bead gels using a solvent that allows the adsorption phenomenon to occur. This paper therefore reports the elution characteristics of a number of heteroatom PAH from Bio-Beads SX-12 gels with benzene, a system in which adsorption occurs [27, 29]. Further, with this system, hydrogen bonding (which can occur in LH-20) should not be a factor in the elutions. The elutions are tabulated and discussed in a manner that emphasizes the contribution of the heteroatom to the gel elution effect while minimizing structural anomalies.

EXPERIMENTAL

Gel filtration chromatography

Four 1.25- × 109-cm Chromatronix LC columns were connected in series and packed with Bio-Beads SX-12 (Bio-Rad Laboratories, Richmond, California) in benzene. The total length of the wet gel bed was 400 cm (ca. 200 g of dry beads).

About 0.1—0.5 mg of each standard (Table 1), dissolved in 1 ml of benzene, was placed onto the gel columns with a 1.0-ml loop injection valve. Benzene was pumped (Chromatronix CMP-3 pump) at a constant flow of 120 ml h^{-1}; 8-ml fractions were collected, evaporated to dryness with a stream of nitrogen, and analyzed by gas chromatography (g.c.). As many as eight g.c. resolvable standards were run at one time on the columns. The percentage distribution (based on total g.c. peak area counts) of individual compounds was calculated for each gel fraction. The elution of several volatile compounds (*N*-methyl-

TABLE 1

Gel filtration elution of PAH and their heterocyclic analogues on Bio-Beads SX-12 with benzene

Compound	Percent distribution											
	Fraction number[a]											
	40	41	42	43	44	45	46	47	48	49	50	51
Naphthalenes and quinolines												
Naphthalene			0.4	25.7	53.7	19.3	0.9					
Quinoline			9.4	47.4	37.8	5.3	0.1					
Isoquinoline			0.4	6.5	48.9	38.3	5.9					
1-Methylnaphthalene		0.4	4.6	25.6	51.1	16.4	1.5					
2-Methylnaphthalene			3.8	44.6	40.8	9.2	1.5					
4-Methylquinoline			3.1	52.6	43.0	1.4						
6-Methylquinoline			7.0	63.6	29.0	0.3						
7-Methylquinoline		0.2	25.2	55.4	17.4	1.5						
Aza PAH												
Phenanthrene			0.4	1.1	22.2	45.0	27.3	4.7	0.3			
3,4-benzoquinoline				6.3	37.4	36.9	17.1	2.3				
5,6-Benzoquinoline				1.4	24.7	46.4	22.7	4.2	0.5			
7,8-Benzoquinoline				1.9	14.2	43.8	31.9	7.3	0.9			
Pyrene					0.1	4.3	26.9	42.1	21.7	4.9	0.1	
1-Azapyrene					1.7	11.7	34.1	31.5	11.7	6.4	1.5	
1,2-Benzanthracene			2.6	22.0	35.1	27.1	11.0	2.1				
3,4-Benzacridine				10.6	46.7	34.7	8.1					
1,2,5,6-Dibenzanthracene				0.5	10.4	34.4	35.8	15.6	3.3			
1,2,5,6-Dibenzacridine			0.6	8.3	28.2	34.8	19.9	6.6	1.7			
Anthracene-type heterocyclics												
Anthracene			0.2	11.2	46.9	34.4	6.7	0.5				
Acridine			1.5	26.4	48.5	19.2	4.1	0.3				
9,10-Dihydroanthracene	13.5	45.4	35.7	5.2	0.3							
Xanthene	0.1	0.6	20.0	51.1	24.1	3.4	0.2					
9,10-Dihydroacridine					6.3	39.5	37.9	13.7	2.6			
Indene-type heterocyclics												
Indene		2.2	40.6	45.6	10.1	1.4						
Benzofuran				0.4	25.3	51.3	20.1	2.6	0.4			
Benzothiophene					0.4	16.9	44.7	29.5	8.4	1.7		
Indole						0.7	11.3	44.0	34.0	8.7	1.3	
1-Methylindene			13.0	54.4	28.5	4.1						
N-Methylindole			2.6	24.6	48.2	19.5	5.1					
3-Methylindole					0.8	24.6	50.2	23.1	1.3			
2,5-Dimethylindole			1.4	36.1	49.2	13.0	0.3					
2,3,5-Trimethylindole		0.9	28.9	56.0	13.1	1.1						
Fluorene-type heterocyclics												
Fluorene		0.8	24.7	52.0	20.0	2.3	0.1					
Dibenzofuran			6.9	46.2	38.5	7.8	0.5					
Dibenzothiophene					19.9	46.0	32.2	1.9				

TABLE 1 (continued)

Compound	Percent distribution											
	Fraction number[a]											
	40	41	42	43	44	45	46	47	48	49	50	51
Carbazole					0.1	3.9	28.7	44.0	19.8	3.5		
4-Azafluorene	1.3	28.3	53.7	15.3	1.2	0.1						
1-Methylfluorene		15.5	53.3	27.8	3.3							
9-Methylfluorene	2.4	34.5	41.7	14.3	5.4	1.8						
N-Ethylcarbazole	3.5	20.9	45.3	25.6	4.6							
2-Methylcarbazole					0.0	21.8	47.3	24.9	4.4	0.6		
Benzo(b)fluorene		1.1	10.9	44.8	34.1	7.5	1.4					
Benzo(b)naphtho-(2,3-d)-furan				4.6	34.9	51.2	9.3					
Benzo(b)carbazole							8.3	14.6	22.1	32.0	19.6	3
Dibenzo(c,g)-carbazole								3.8	18.5	55.5	18.3	3

[a]Number of 8-ml fractions from point of injection.

indole, indene, etc.) was monitored by a u.v. monitor (Chromatronix Model 230) at 280 nm.

Gas chromatography

A Hewlett-Packard Model 5750 gas chromatograph was equipped with a 15-ft. × 0.125-in. i.d. stainless steel column packed with 5% Dexsil 300 GC on 100/120 mesh Chromosorb W-AW (temperature program: 100—325°C at 8° min^{-1}; He flow, 48 ml min^{-1}; injector, 290°C; flame detector, 350°C). Peak areas were determined with an Autolab System IV electronic integrator (Spectra Physics).

RESULTS AND DISCUSSION

The gel elution volumes of a number of aza-arenes containing an sp^2 nitrogen in the aromatic ring were compared with those of their PAH analogues. The data in Table 1 show that quinoline, isoquinoline, benzoquinolines, acridine, benzacridine, dibenzacridine, and 1-azapyrene have almost the same elution volumes as their PAH analogues. Thus, all of these nitrogen compounds are adsorbed by the gel in a manner and degree similar to the corresponding PAH. The slightly earlier elution of these aza-arenes, relative to the PAH analogues, agrees with the fact that the π-electron densities in these nitrogen compounds are slightly lower than in the PAH. The lower density is due to the drift of electrons toward and partial localization of the charge on the nitrogen [31].

The gel elution properties of a number of oxygen, sulfur, and nitrogen analogues of PAH containing sp^3 carbon are also listed in Table 1 and are

compared with indene, fluorene, and anthracene. The order of elution found in several series is carbon-, oxygen-, sulfur-, and nitrogen—PAH, as represented by indene, benzofuran, benzothiophene, and indole. This order of elution also held for their benzo and dibenzo analogues (Table 1). The order of elution reflects the ability of the different molecules in the series to form a π—π complex with the polystyrene gel. Although other factors may be considered (i.e., electronegativity, solvolysis, dimerization, hydrogen bonding, hyperconjugation, etc.), the degree of π-complexation apparently is proportional to the amount of ring current or π-electron delocalization in the molecule. Calculations have shown thiophene to have a slightly greater ring current than furan [32]. Thus, benzothiophene would be expected to elute after benzofuran, as was observed. Presumably, the *d*-orbitals on sulfur enhance the π-electron density of the S—PAH. The extremely late elution of the nitrogen compounds (indole, carbazole, etc.) illustrates the availability of the lone pair of electrons on nitrogen for significant delocalization into the π-system and results in an increased π-electron density of the molecule. As expected from the above discussion, 4-azafluorene with the sp^2-hybridized nitrogen was eluted slightly earlier than fluorene.

The data show that the methyl effect on elution found for PAH [29] was also valid for methylquinolines, indoles, and carbazoles. The data for the mono-, di-, and trimethylindole series show the overall effect of decreasing elution volume with increasing alkylation. The extreme decrease in elution of *N*-methylindole and *N*-ethylcarbazole as compared with those of the parent compounds indicates a decreased amount of π-complex formation on the gels. This may be due to a decrease in the availability of the nitrogen π-electrons for delocalization rather than a steric effect because methylation at other positions of indole and carbazole (i.e., 3-methylindole and 2-methylcarbazole) produces a smaller but normal decrease in elution time relative to the parent.

The gel elution concepts discussed above have been applied to the characterization and identification of N—PAH in cigarette smoke condensate [4, 33] and should be applicable to other POM sources in the environment.

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DETERMINATION OF PRIMARY AMINES BY MEANS OF FLUORESCENT SCHIFF BASE DERIVATIVES

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SUMMARY

The formation of Schiff bases from the reaction of primary amines and several aromatic aldehydes has been studied. In many cases the Schiff bases were too unstable or feebly-fluorescent to be of analytical value. 1-Pyrenealdehyde and 2-fluorenealdehyde, however, were found to be suitable fluorigenic reagents for primary aliphatic amines, forming Schiff bases that were very stable and intensely fluorescent in acidic ethanol. The derivatives of 1-pyrenealdehyde could be detected at concentrations less than 1 ng ml^{-1} in pure solution. Derivatives of 1-pyrenealdehyde could be readily produced by reactions at the surface of a t.l.c. plate. Combination of this approach with a simple deproteinizing procedure permitted analysis for nanogram quantities of primary amines in blood serum.

The sensitivity and selectivity of fluorimetric methods have encouraged their widespread use in the analysis of organic materials, and many methods for generating strongly fluorescent derivatives of feebly- or non-fluorescent compounds have been devised. In particular, several routes for the production of fluorescent derivatives of primary amines are well-established. Amongst the commonest are the use of the labels dimethylaminonaphthalene sulphonyl chloride (dansyl chloride) [1], fluorescamine (4-phenylspiro[furan-2-(3H), 1-phthalan]-3,3′-dione) [2] and NBD chloride (4-chlor-7-nitrobenz-2,1,3-oxadiazole) [3]. These reagents are not without their disadvantages. Thus, dansyl chloride and NBD chloride react with secondary as well as primary amines, and also with other functional groups; fluorescamine, which is non-fluorescent itself and reacts instantly and specifically with primary amines, the excess of reagent being hydrolysed to non-fluorescent products, is costly and its fluorescent derivatives are often not stable [4, 5]. Lisy and Gerstein [6] described a method for the determination of tryptamine in the nanogram range. The tryptamine reacted with benzaldehyde to produce a Schiff base which gave an intensely fluorescent product on treatment with acid. Spatz and Spatz [7] used *p*-dimethylaminocinnamaldehyde as the fluorigenic

reagent for a number of primary amines. Aldehyde derivatives of polycyclic aromatic hydrocarbons have previously been used to determine nitrosamines, after reduction of the latter to hydrazines [8].

The availability of a number of fluorigenic reagents capable of reacting with specific functional groups of non-fluorescent compounds has permitted the development of analytical methods which combine the sensitivity of fluorimetry with the selectivity of a separation technique. The combination of fluorimetry and thin-layer chromatography (t.l.c.-fluorimetry) has the additional advantages of simplicity and the capacity for the study of several samples simultaneously [9]. Fluorescent derivatives can be formed at several different stages of a t.l.c. fluorimetric analysis. Thus fluorescamine derivatives of primary amines have been produced by reacting the amines with the reagent immediately before applying the samples to the t.l.c. plate [10]; by reaction at the origin of the t.l.c. plate immediately before development [11, 12]; and by reaction on the completely developed plate [13].

In the present paper the potential value of a number of aromatic aldehydes as fluorigenic reagents for primary amines is assessed, and it is shown that 2-fluorenealdehyde and, in particular, 1-pyrenealdehyde are valuable reagents. Optimum reaction conditions are discussed, and it is shown that the fluorescent derivatives can be conveniently formed at the surface of a t.l.c. plate. The combined t.l.c. fluorimetric analysis is shown to be suitable for the rapid and simple determination of nanogram quantities of amines, both in pure solution and in blood serum.

EXPERIMENTAL

Chemicals

Benzaldehyde, *m*- and *p*-hydroxybenzaldehydes, 2,4- and 2,5-dihydroxybenzaldehydes and *p*-dimethylaminobenzaldehyde were obtained from British Drug Houses Ltd.; *o*-hydroxybenzaldehyde (salicylaldehyde) and 3-indolealdehyde from Sigma Chemical Co.; and 1-naphthaldehyde, 2-hydroxy-1-naphthaldehyde, 9-anthraldehyde, 9-phenanthraldehyde, 2-fluorenealdehyde, and 1-pyrenealdehyde from Aldrich Chemical Co. These reagents were purified either by vacuum distillation or by recrystallization to sharp melting points from benzene—ethanol mixtures or from petroleum ether. Amines were obtained from various commercial sources and were also purified by distillation or recrystallization. Ethanol (James Burroughs Ltd., AR grade) was re-distilled before use as a solvent for most fluorimetric studies. Other reagents were AnalaR or equivalent grade.

Preparation of Schiff bases

Pure samples of the Schiff bases were prepared in the usual way [14]. Between 0.25 and 0.50 g of the amine was dissolved in 10 ml of benzene. A stoichiometric amount of the aldehyde was also dissolved in 10 ml of benzene. The two solutions were mixed and refluxed for 1—2 h in a round-

bottomed flask fitted with a Dean and Stark attachment. After removal of the water from the condensation reaction and partial evaporation of the benzene, the flask was cooled in ice for 30 min. The solid product was filtered through a Buchner funnel, and washed with cold dry benzene. It was recrystallized to a sharp melting point from benzene. Since tyramine and *o*-aminobenzoic acid are only slightly soluble in benzene, ethanol was used as the solvent in these cases. Refluxing proceeded for up to 3 h and the Dean and Stark attachment was not used. The purified Schiff bases were characterized by the melting points and by their infra-red spectra. The latter were obtained from Nujol mulls with a Pye-Unicam SP200G grating spectrometer; the Schiff bases showed characteristic —CH=N— stretching frequencies between 1625 and 1655 cm^{-1}. In one case, 1-pyrenealdimine-benzylamine, the structure of the product was further verified by mass spectrometry and n.m.r. In some experiments, Schiff bases were formed by reaction on a t.l.c. plate (see below).

Fluorescence measurements

Fluorescence measurements were performed on a Baird-Atomic (Braintree, U.K.) Fluoricord spectrofluorimeter fitted with a 150-W xenon arc lamp. Uncorrected excitation and emission spectra were recorded on a Bryans 27000 recorder. Samples were characterized in 10-mm square silica cuvettes at concentrations of not more than 5 $\mu g\ ml^{-1}$. The detection limit was defined in each case as the concentration of solute yielding a signal exceeding the background signal by two standard deviations.

Thin-layer chromatography

Thin-layer chromatography was carried out on flexible t.l.c. plates coated with 250-μm layers of silica gel G60 (Merck). Samples (10 μl) were applied with disposable micropipettes. Eluting solvents were re-distilled and dried over molecular sieves before use. Ascending development was carried out in glass chromatography tanks lined with filter paper. Fluorescence densitometry of developed t.l.c. plates was done with a Vitatron TLD 100 densitometer (Fisons Scientific Apparatus Ltd., Loughborough), fitted with a mercury lamp and an interference filter with maximum transmission at 510 nm. Fluorescence emission spectra of compounds separated by t.l.c. were studied by wrapping the flexible t.l.c. plates round a brass drum which was inserted into the sample compartment of the Fluoricord fluorimeter in place of the usual sample holder. The drum, which could be rotated by a small electric motor, was positioned so that the sample spots could be brought in turn into the light beam. This device is a simplified version of the thin-layer phosphorimeter previously developed in this laboratory [15].

Human serum samples were deproteinized by mixing them with nine times their volume of ethanol and warming to 60°C for 10 min. The flocculated proteins were removed by centrifugation and the deproteinized samples applied directly to t.l.c. plates, prior to derivatization.

RESULTS

Benzaldehyde and its derivatives proved to be of no general value as fluorigenic reagents. In most cases, the Schiff bases formed were either non-fluorescent, or appeared to fluoresce relatively feebly at wavelengths similar to those of the parent aldehyde. The products formed by reacting 1-naphthaldehyde, 2-hydroxy-1-naphthaldehyde and 3-indole-1-aldehyde were likewise only weakly fluorescent in ethanol solution; in ethanol that had been acidified with dry HCl (see below) these Schiff bases were rapidly hydrolysed. 9-Anthraldehyde yielded Schiff bases that were moderately fluorescent, but were again unstable in acidic solution. Schiff bases derived from 9-phenanthraldehyde were not fluorescent in neutral ethanol, and only weakly fluorescent in acidic ethanol.

2-Fluorenealdehyde, itself non-fluorescent in neutral ethanol, and only weakly fluorescent in acidic ethanol, was found during preliminary studies to yield Schiff bases that fluoresced strongly in acidic ethanol. 1-Pyrenealdehyde is strongly fluorescent in both neutral and acidic ethanol: the corresponding Schiff bases were moderately fluorescent in neutral ethanol, and strongly fluorescent in acidic ethanol. It is thus evident that 2-fluorenealdehyde and 1-pyrenealdehyde were the most promising fluorigenic reagents and their properties and those of their derivatives were studied in detail.

2-Fluorenealdehyde derivatives

Table 1 shows the properties of 2-fluorenealdehyde, and of the typical Schiff base derivative formed with benzylamine in a variety of neutral and

TABLE 1

Fluorescence properties of 2-fluorenealdehyde and its benzylamine derivative in various solvents

Solvent	2-Fluorenealdehyde		2-Fluorenealdehyde benzylamine derivative		
	λ_{ex}(nm)	λ_f(nm)	λ_{ex}(nm)	λ_f(nm)	Relative intensity[a]
Acetone	NF[b]			NF	
Acetone—HCl[c]	NF		359	445	1.07
Chloroform	NF		363	420	< 0.01
Chloroform—HCl[c]	NF		367	430	1.18
Ethyl Acetate	NF			NF	
Ethyl Acetate—HCl[c]	NF		360	435	0.78
1-Butanol	NF			NF	
1-Butanol—HCl[c]	310	340	363	435	0.68
Ethanol	NF			NF	
Ethanol—HCl[c]	305	335	361	435	1.00

[a]Intensities compared with those in ethanol—HCl. [b]NF, non-fluorescent. [c]Solvents acidified by passing dry HCl gas through them.

acidified solvents. The Schiff base was only fluorescent in the acidified solvents, and its optimum excitation and fluorescence wavelengths were longer than those of the parent aldehyde. Although it did not always give the optimum intensity, ethanol (acidified with dry HCl) was chosen as the solvent for routine work, in view of its very low luminescence background. Table 2 shows the properties of a number of Schiff base derivatives obtained with 2-fluorenealdehyde. Aromatic amines formed derivatives which were only feebly fluorescent and were quickly hydrolysed, and hydrazines, e.g. phenelzine, gave non-fluorescent derivatives, but primary aliphatic amines formed intensely fluorescent derivatives. These derivatives were stable in acidic ethanol over a period of several weeks. Most of the derivatives could be detected at levels of about 1 ng ml^{-1} in pure solution. The tyramine derivative was much less fluorescent than that of the related molecule phenylethylamine, and the product from dopamine was even less fluorescent. In most cases a plot of fluorescence intensity against concentration was linear over at least three orders of magnitude: at concentrations greater than about 1 μg ml^{-1}, inner filter effects in the strongly absorbing solution caused negative deviations from linearity.

1-Pyrenealdehyde derivatives

The results in Table 3 show that 1-pyrenealdehyde was itself fluorescent in a variety of neutral and acidified solvents. The Schiff bases formed with primary aliphatic amines were again intensely fluorescent in acidic ethanol with typical excitation and emission wavelengths of ca. 450 nm and ca. 500 nm respectively, substantially longer than those of the parent aldehyde (Fig. 1). The fluorescence intensity was sufficient to permit the determination of subnanogram concentrations, with linear fluorescence—concentration plots up to at least 1 mg ml^{-1}, although tyramine again gave only a feebly-fluorescent derivative (Table 4). In contrast to the derivatives of 2-fluorenealdehyde, the Schiff bases formed by 1-pyrenealdehyde also fluoresced in certain neutral

TABLE 2

Fluorescence properties of 2-fluorenealdehyde and its derivatives in acidic ethanol

Compound	ϵ ($l\ cm^{-1}\ mol^{-1}$)	λ_{ex} (nm)	λ_f (nm)	Relative intensity[a]	Limit of detection (ng ml^{-1})
2-Fluorenealdehyde	12,000	305	335	—	—
-benzylamine	39,000	368	435	1.00	1
-2-phenylethylamine	92,000	360	431	0.97	1
-cyclohexylamine	48,000	358	427	1.08	1
-tyramine	39,000	353	392	0.01	100
-histamine	54,000	360	433	0.49	2
-amphetamine	46,000	360	428	3.16	0.4

[a]Intensity compared with that of the benzylamine derivative.

TABLE 3

Fluorescence properties of 1-pyrenealdehyde and its benzylamine derivative in various solvents

Solvent	1-Pyrenealdehyde		1-Pyrenealdehyde-benzylamine derivative		
	λ_{ex}(nm)	λ_f(nm)	λ_{ex}(nm)	λ_f(nm)	Relative intensity[a]
Acetone	348	394	358	412	< 0.01
Acetone—HCl	348	394	438	512	0.98
Chloroform	395	426	360	402	0.38
Chloroform—HCl	395	426	455	505	1.02
Ethyl Acetate	348	392	NF		—
Ethyl Acetate—HCl	348	392	436	504	0.69
1-Butanol	340	400	358	406	0.78
1-Butanol—HCl	340	400	447	506	0.93
Ethanol	394	452	355	400	1.00
Ethanol—HCl	338	399	445	508	1.00

[a]Intensities in neutral solvents compared with that in neutral ethanol: intensities in acidified solvents compared with that in acidified ethanol.

TABLE 4

Fluorescence properties of 1-pyrenealdehyde and its derivatives in acidic ethanol

Compound	ϵ (l cm^{-1} mol^{-1})	λ_{ex} (nm)	λ_f (nm)	Relative intensity[a]	Limit of detection (ng ml^{-1})
1-Pyrenealdehyde	29,500	338	399	—	—
-benzylamine	35,500	450	505	1.00	0.1
-2-phenylethylamine	33,500	450	505	0.95	0.1
-cyclohexylamine	30,000	440	500	1.21	0.1
-tyramine	14,000	448	500	0.01	10
-histamine	34,500	450	503	0.91	0.1
-amphetamine	52,500	450	501	1.50	0.07

[a]Intensity compared with that of the benzylamine derivative.

solvents, but the fluorescence was considerably feebler than in acidic solution and at lower wavelengths (typically λ_{ex} = 360 nm, λ_f = 405 nm). No significant changes of fluorescence intensity were observed on storing the derivatives in solution over many weeks.

A large excess (ca. 1000:1 molar ratio) of 1-pyrenealdehyde was required to produce the maximum fluorescence intensity in the reaction with benzylamine in 1-butanol: very similar results were obtained when ethanol was the solvent, and also when 2-fluorenealdehyde was used as the fluorigenic reagent. In addition the formation of the Schiff base was not complete even

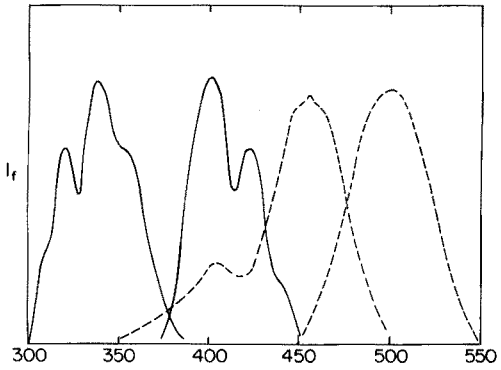

Fig. 1. Uncorrected excitation and fluorescence spectra of 1-pyrenealdehyde (——) and a typical amine derivative (----------) in ethanol acidified with dry HCl.

after refluxing the amine for 5—6 h with 1-pyrenealdehyde in 1-butanol, although analytically useful fluorescence could be observed after acidifying a mixture refluxed for considerably shorter periods.

Attempts to produce more convenient reaction conditions revealed that Schiff base formation occurred readily on the surface of a t.l.c. plate. In a typical experiment 10 μl of a solution of an amine in ethanol were applied to the chromatographic surface with a micropipette. After a few minutes, an equal volume of 1-pyrenealdehyde in ethanol or benzene was added: up to a thousand-fold excess of the aldehyde could be used without significantly increasing the background fluorescence, or causing tailing of the chromatographic zones, in the final determination of the Schiff base. The plate was then sprayed with 1-butanol acidified with dry HCl and placed in an oven at 70°C for 1 h. The chromatogram was developed with 1-butanol:ethyl acetate (both solvents saturated with dry HCl), 1:4 v/v, except in the analysis for histamine, when HCl-saturated 1-butanol was the developing solvent. The developed plates were evaluated as described above. Serum samples were de-proteinized as already described before analysis: Table 5 shows that recoveries of amphetamine after using warm ethanol as a protein precipitant were very high.

Figure 2 shows that formation of the fluorescent derivatives was apparently completed within 30—40 min in the cases of 2-phenylethylamine, amphetamine and cyclohexylamine. The fluorescence spectra of the derivatives examined on t.l.c. plates were identical to those of the same derivatives in alcoholic solution, all the derivatives having fluorescence maxima between 490 and 500 nm (Fig. 3.).

Experiments with solutions of pure amines showed that subnanogram concentrations could be detected (Table 6). The detection limits for amines in blood serum were slightly inferior, though still at the nanogram level, and linear analytical plots were obtained (Fig. 4). In all cases, the fluor-

TABLE 5

Recovery of amphetamine from serum after deproteinization and t.l.c. fluorimetry of its 1-pyrenealdehyde derivative

Amphetamine concentration ($\mu g\ ml^{-1}$)	Fluorescence intensity[a] (arbitrary units)		Recovery (%)
	Spiked serum	Standard	
100	139.2	138.0	100.7
10	135.0	140.5	96.1
1	52.0	55.0	91.2

[a]Mean of four values in each case.

TABLE 6

Chromatographic properties and detection limits of some primary amines determined by t.l.c. fluorimetry of their 1-pyrenealdehyde derivatives

Amine	R_F	Detection limits (ng/spot)	
		Pure amine	In serum
Amphetamine	0.20	0.2	1.0
2-phenylethylamine	0.27	0.1	1.0
Tyramine	0.21	2.0	2.0
Histamine	0.12	4.0	5.0
Benzylamine	—	0.5	—
Cyclohexylamine	—	0.5	—
3-Phenylpropylamine	—	0.5	—

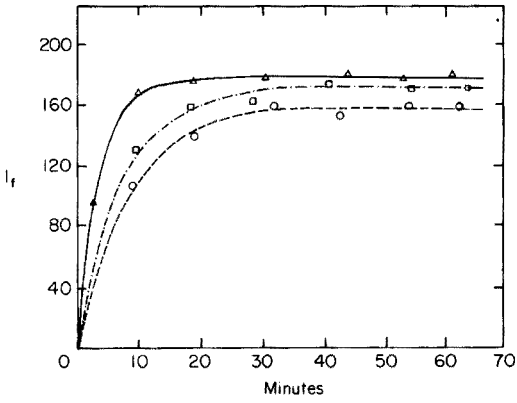

Fig. 2. The reaction between 1-pyrenealdehyde and various amines on the surface of a silica gel plate in acidic conditions. The aldehyde—amine ratio was 1000:1, and the fluorescence intensity was measured at 510 nm in each case. (△) 2-Phenylethylamine; (□) amphetamine; (○) cyclohexylamine.

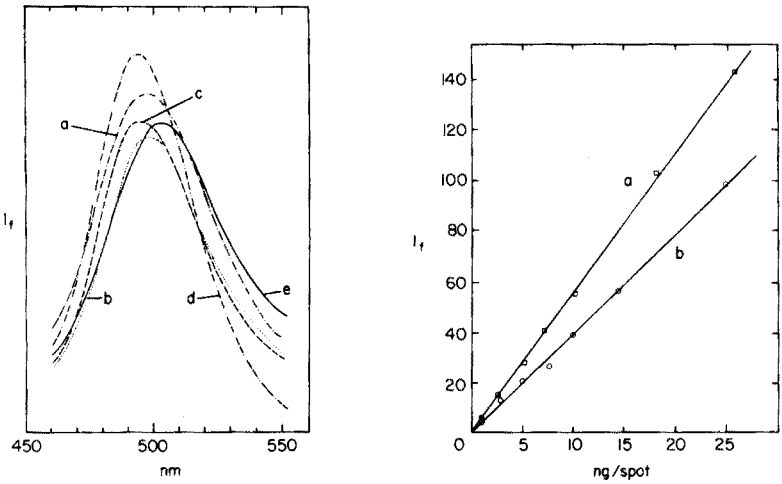

Fig. 3. Fluorescence spectra of the Schiff bases formed from 1-pyrenealdehyde and primary amines on silica gel surfaces in acidic conditions. The excitation wavelength was 440 nm in each case. (a) Histamine; (b) benzylamine; (c) amphetamine; (d) 2-phenylethylamine; (e) cyclohexylamine.

Fig. 4. Analytical curves for the determination of amphetamine and histamine in serum. Deproteinized serum was treated with 1-pyrenealdehyde (0.04 g ml^{-1}) on acidified t.l.c. plates and the reaction took place for 60 min at 70°C. The fluorescence was measured at 510 nm.

escence of the Schiff base derivatives on t.l.c. plates remained stable over periods of several weeks, provided that the plates were stored in the absence of water vapour: however, the Schiff bases were hydrolysed within 30 min in an ordinary laboratory atmosphere.

DISCUSSION

The reactions between amines and carbonyl compounds that lead to the formation of the Schiff bases (imines) have been frequently studied and discussed in several reviews [6]. Such reactions involve two reversible steps, the first involving the formation of an unstable tetrahedral carbinolamine, and the second the dehydration of this intermediate to form the imine:

$$R_1NH_2 + R_2R_3CO \rightleftharpoons \left[\begin{array}{c} R_2 \quad NHR_1 \\ \diagdown \quad / \\ C \\ / \quad \diagdown \\ R_3 \quad OH \end{array}\right] \rightleftharpoons R_2R_3C{=}NR_1 + H_2O$$

The Schiff bases of aliphatic aldehydes are unstable and polymerize readily [17] whereas those derived from aromatic aldehydes are stabilized by their

effective conjugation systems [18]. For any given aldehyde the rate of Schiff base formation increases with the basicity of the amine; primary aliphatic amines thus form Schiff bases more rapidly than anilines and other weak bases [19]. As secondary amines do not yield Schiff bases (alkylidenediamines are formed instead [20]), the fluorigenic reactions described in this work would seem to be effectively specific for primary aliphatic amines. The value of the procedure will also depend in practice on the stabilities of the Schiff bases, and in particular their resistance to hydrolysis. This hydrolytic reaction has been studied by Cordes and Jencks [21] and others, and is both acid- and base-catalysed. In the present method, the removal of water by the Dean and Stark procedure was essential to obtain a reasonable yield of the Schiff bases, and many of the products were found to be extremely susceptible to hydrolysis.

Acidification of the solutions with dry HCl substantially enhanced the fluorescence of many of the aldehydes, probably through acetal formation. Only 1-pyrenealdehyde showed appreciable fluorescence in neutral ethanol; Bredereck et al. [22] previously stated that 3-pyrenealdehyde was strongly fluorescent in polar solvents. Acidification of the Schiff base derivatives produced a substantial bathochromic shift in their absorption maxima, an effect noted by earlier workers [23], and also in their fluorescence maxima. In the cases of 2-fluorenealdehyde and 1-pyrenealdehyde derivatives, the shifts were found to be so great that the excitation maxima of the Schiff bases were at higher wavelengths than the fluorescence maxima of the parent aldehydes in the same solvent. This property enhances the value of these aldehydes as fluorigenic reagents, because large excesses of the aldehydes can be used to ensure complete derivatization of the amines without appreciably affecting the background fluorescence at the emission wavelengths of the products. In the absence of water, the acidified Schiff bases derived from polycyclic aromatic aldehydes retain their fluorescence over periods of several weeks: charge delocalization effects may again be responsible for their stability.

Comparison of 2-fluorenealdehyde and 1-pyrenealdehyde as fluorigenic reagents indicates that the latter is generally superior. Although they have generally lower molar absorptivities, derivatives of 1-pyrenealdehyde can be detected at concentrations approximately one order of magnitude lower than those of 2-fluorenealdehyde. This may in part reflect the higher quantum yields of the 1-pyrenealdehyde derivatives, although the lower background fluoresecnce at ca. 500 nm may also contribute to the superior detection limit. The aromatic group in tyramine drastically reduces the quantum yields of the derivatives of both aldehydes, and the dopamine derivatives showed an even feebler fluorescence.

In many respects, 1-pyrenealdehyde is an ideal fluorigenic reagent: it is readily and cheaply available in a state of reasonable purity, it is effectively specific for primary aliphatic amines, and its derivatives are stable and intensely fluorescent at wavelengths where the sensitivities of conventional fluorimeters are good but the background fluorescence from biological samples is often

relatively low. Like other less specific labels such as dansyl chloride it requires fairly rigorous conditions for complete reaction with the sample molecules: but the formation of the fluorescent Schiff bases proceeds more rapidly at the surface of a t.l.c. plate than in solution. In conjunction with the convenient and rapid ethanol deproteinization step, nanogram quantities of primary amines in diluted blood serum can readily be determined. This permits the detection of $\mu g\ ml^{-1}$ concentrations of the amines in a few μl of serum without the use of an evaporation step or other means of concentration. Nor is a solvent extraction step required.

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FLUORIMETRIC DETERMINATION OF AROMATIC ALDEHYDES WITH 2,2′-DITHIOBIS(1-AMINONAPHTHALENE)

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SUMMARY

A sensitive fluorimetric method for the determination of aromatic aldehydes is based on their reaction in dilute sulfuric acid with a new reagent, 2,2′-dithiobis(1-aminonaphthalene) in the presence of tri-n-butylphosphine, sodium sulfite and sodium phosphite at ambient temperature. The fluorescences produced are fairly characteristic of individual aldehydes. The method is extremely selective for aromatic aldehydes and very sensitive, especially for *p*-hydroxybenzaldehyde, *o*-methoxybenzaldehyde, *p*-methoxybenzaldehyde and *p*-tolualdehyde which can be determined at concentrations of as little as 3—5 ng ml^{-1}.

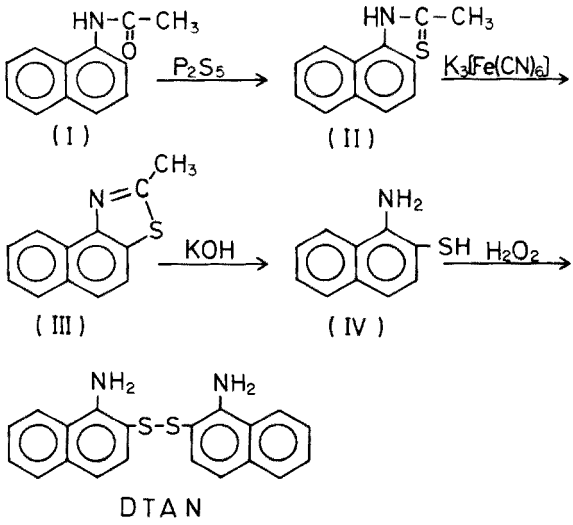
Numerous fluorimetric methods have been developed for the determination of aliphatic aldehydes [1, 2], but for aromatic aldehydes only two methods, based on reactions in acidic media with 2-aminothiophenol [3] and with 1,2-diaminonaphthalene [4], have been proposed. In the course of studies of the assay of dopamine β-hydroxylase in biological samples, 2,2′-dithiobis-(1-aminonaphthalene) was found to react extremely selectively in acidic medium with aromatic aldehydes, even at low ambient temperature, in the presence of some reducing substances to give a compound which fluoresces intensely. *p*-Hydroxybenzaldehyde was employed as a model compound to establish suitable reaction conditions for a more general analytical method.

EXPERIMENTAL

Reagents and solutions

All chemicals were of reagent grade (Japanese Industrial Standards), unless otherwise specified. Double-distilled water was used throughout.

2,2′-Dithiobis(1-aminonaphthalene) (DTAN). DTAN is stable in air and in daylight at room temperature; it may form a complex with transition metal ions which should therefore be absent. The reagent was synthesized by the Jacobson method [5] with some modifications (see Scheme) as follows. Mix 25 g of 1-acetamidonaphthalene (I) with 15 g of phosphorus pentasulfide, add 300 ml of xylene and reflux with stirring for 10—15 min. After removal of the solvent, extract the resulting residue with 200 ml of



5% sodium hydroxide solution and acidify the extract with 20% sulfuric acid. Filter the precipitate of 1-thioacetamidonaphthalene (II) thus formed and recrystallize from ethanol (colorless prisms, m.p. 111°C; yield, ca. 17 g). Dissolve 10 g of (II) in 200 ml of 5% sodium hydroxide solution, add 20% potassium hexacyanoferrate solution dropwise and stir on a magnetic stirrer for 30 min. Filter the resulting precipitate of 2-methylnaphtho-[1,2-d]-thiazole (III) and recrystallize from ethanol (colorless prisms, m.p. 95°C; yield, ca. 8 g). Place 2 g of (III) in a pyrex tube (15 mm diam., 250 mm long), add 6 g of powdered potassium hydroxide and 4 ml of ethanol, and seal the tube. Heat the tube in an oil bath at 190—195°C for 4 h. Immediately after opening the tube, extract the resulting crude crystals of 1-amino-2-thionaphthol (IV), which is unstable in air, with about 200 ml of 5% sodium hydroxide solution. Add ca. 100 ml of 10% hydrogen peroxide solution, extract the resulting yellow mass of crude DTAN with benzene, and dry the extract over anhydrous sodium sulfate. In order to purify DTAN, pour the concentrated benzene extract onto a column packed with a small amount of neutral alumina and elute with benzene. Concentrate the eluate almost to dryness, and recrystallize from a mixture of benzene and cyclohexane (1:4) to yellow needles (m.p. 131°C; yield 1.7 g).

Aldehydes. Commercial aldehydes were purified just before use by distillation or recrystallization in the usual manner.

DTAN solution. Dissolve 40.0 mg of DTAN in 6.0 ml of methanol, add 1.0 ml of a solution (80 mg ml^{-1}) of tri-n-butylphosphine in methanol and 25.0 ml of 30% sulfuric acid, and dilute with water to 100 ml. The solution is stable for at least one week when stored in a refrigerator.

β-Mercaptoethanol solution. Add 5.0 ml of distilled β-mercaptoethanol to 33.5 ml of 30% sulfuric acid and dilute with water to 50 ml. Use within 3 days.

Aldehyde solutions. Prepare aqueous solutions. Water should be degassed by boiling for several min to prevent oxidation of aldehyde by dissolved oxygen.

Apparatus

Fluorescence spectra and intensities were measured at a constant temperature of 25°C with a Hitachi MPF-4 Spectrofluorimeter with a xenon arc-lamp and quartz cells of 10 × 10 mm optical path length. In this fluorimeter, the slit-widths in terms of wavelength were set at 10 nm in both the exciter and analyser. A daily check of sensitivity was made by measuring the fluorescence intensity of a solution (0.1 or 0.5 μg ml^{-1}) of quinine sulfate dihydrate in 0.05 M sulfuric acid at an emission wavelength of 450 nm with excitation at 350 nm. The fluorescence spectra, and excitation and emission maxima are uncorrected.

Procedure

Place 1.0 ml of the test solution in a test tube, add 0.5 ml of freshly prepared aqueous 0.05% (w/v) sodium sulfite solution and 0.5 ml of freshly prepared aqueous 15.0% (w/v) sodium phosphite pentahydrate solution and warm in a water bath at 37°C for 5 min. Then add 2.0 ml of DTAN solution and allow to stand at 37°C for exactly 30 min to develop to fluorescence. Add 1.0 ml of β-mercaptoethanol solution to stop the reaction. Prepare a reagent blank by treating 1.0 ml of water in the same manner. Measure the fluorescence intensities of the test and blank at the emission maximum wavelength with irradiation at the excitation maximum (see Table 1). Read the concentration of aldehyde from a calibration curve prepared in the usual manner.

RESULTS AND DISCUSSION

Determination of p-*hydroxybenzaldehyde*

The excitation and emission maxima for the product from *p*-hydroxybenzaldehyde occur at 372 and 460 nm, respectively (Fig. 1). On irradiation at this excitation maximum, a very weak fluorescence of the reagent blank is observed (Fig. 1); the intensity is only 0.5% of that given by 5×10^{-6} M *p*-hydroxybenzaldehyde.

Effects of reactant concentrations. With aromatic aldehydes, DTAN gives a fluorescence in the presence of tri-n-butylphosphine or triphenylphosphine in dilute sulfuric or phosphoric acid, but not in alkaline and neutral media. Tri-n-butylphosphine is soluble in dilute sulfuric acid but triphenylphosphine is insoluble in water and in dilute sulfuric acid. Thus, tri-n-butylphosphine was used; its concentration does not affect the fluorescence development over the concentration range 0.4—2.0 mg ml^{-1} in DTAN solution; a concentration of 0.8 mg ml^{-1} was used.

DTAN is difficultly soluble in water, sparingly soluble in dilute sulfuric or phosphoric acid and moderately soluble in methanol, which does not

TABLE 1

Excitation and emission maxima of the fluorescence from aromatic aldehydes and their lower limits of determination (LD)

	Excitation maximum[a] (nm)	Emission maximum[a] (nm)	M[b]	L.D.[c] (ng ml^{-1})
Benzaldehyde	354	473	1×10^{-7}	10
o-Hydroxybenzaldehyde	371	513	2×10^{-7}	30
m-Hydroxybenzaldehyde	365	473	1×10^{-6}	150
p-Hydroxybenzaldehyde	372	460	2×10^{-8}	3
2,4-Dihydroxybenzaldehyde	383	444	1×10^{-7}	10
3,4-Dihydroxybenzaldehyde	380	460	1.5×10^{-6}	150
o-Methoxybenzaldehyde	380	462	2×10^{-8}	3
m-Methoxybenzaldehyde	361	475	1.5×10^{-7}	20
p-Methoxybenzaldehyde	370	462	2×10^{-8}	3
2,3-Dimethoxybenzaldehyde	365	473	1.5×10^{-6}	200
Vanillin	380	460	5×10^{-8}	8
p-Tolualdehyde	360	470	5×10^{-8}	5
o-Chlorobenzaldehyde	360	470	5×10^{-7}	70
p-Chlorobenzaldehyde	360	475	3×10^{-7}	40
p-Nitrobenzaldehyde	380	465	5×10^{-7}	80
p-Dimethylaminobenzaldehyde	360	480	5×10^{-7}	80
p-Sulfamoylbenzaldehyde	359	493	2.5×10^{-7}	50
β-Naphthaldehyde	374	462	2.5×10^{-7}	40
o-Phthalaldehyde	364	453	1.5×10^{-7}	20
Terephthalaldehyde	360	480	5×10^{-7}	70
Furfural	380	475	5×10^{-7}	10

[a] 1.0 ml portions of 1×10^{-6} or 1×10^{-5} M aldehyde solutions were treated according to the procedure. [b] Expressed as a concentration which gives a fluorescence intensity more than twice the blank. [c] Amount of aldehyde in 1.0 ml of sample solution.

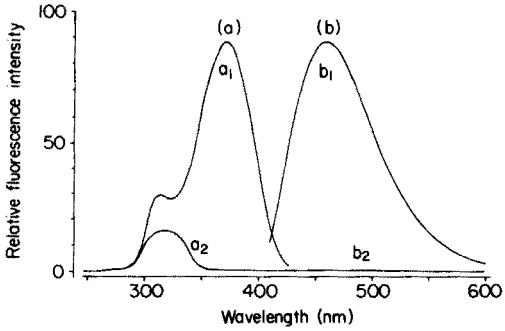


Fig. 1. (a) Excitation (372 nm) and (b) emission (460 nm) spectra of the reaction mixture of *p*-hydroxybenzaldehyde (a_1, b_1) and the reagent blank (a_2, b_2). 1.0 ml of 5×10^{-6} M *p*-hydroxybenzaldehyde was treated according to the procedure.

affect the fluorescence development. Although dilute sulfuric or phosphoric acid can be used, dilute phosphoric acid gives less intense fluorescence and a higher blank value. Therefore, DTAN was dissolved with tri-n-butylphosphine in dilute sulfuric acid containing a small amount of methanol.

The concentration of sulfuric acid in the reagent solution affects the fluorescence development (see Fig. 2). Sulfuric acid concentrations ranging from 6.0 to 8.2% give maximum intensity and 7.5% was selected as optimum.

The fluorescence intensity increases with increasing concentration of DTAN; concentrations higher than 300 μg ml^{-1} in the reagent solution give the most intense and constant fluorescence (Fig. 3); 400 μg ml^{-1} was the optimum selected.

Both sodium sulfite and sodium phosphite accelerate the fluorescence reaction. In their absence, the fluorescence from *p*-hydroxybenzaldehyde was only 11% of the intensity yielded under the recommended conditions, as shown in Fig. 4 (c and f). Without sodium sulfite or sodium phosphite, the fluorescence intensity was 68 or 46% respectively of that given by the prescribed procedure, and so a combination of them was utilized as the accelerator. Sodium sulfite and sodium phosphite pentahydrate provide a maximum and reproducible fluorescence intensity over the concentration ranges of 0.025—0.06% and 12.5—25.0% (w/v), respectively; 0.05 and 15.0% were selected as their optima.

Effect of reaction temperature and time. The fluorescence reaction occurs at 0°C; higher temperature develops the fluorescence more rapidly (Fig. 4). Above 60°C, however, the fluorescence development is retarded slightly. The fluorescence intensities at various temperatures reach maxima after heating for more than 120 min, but a temperature of 37°C, which was easily

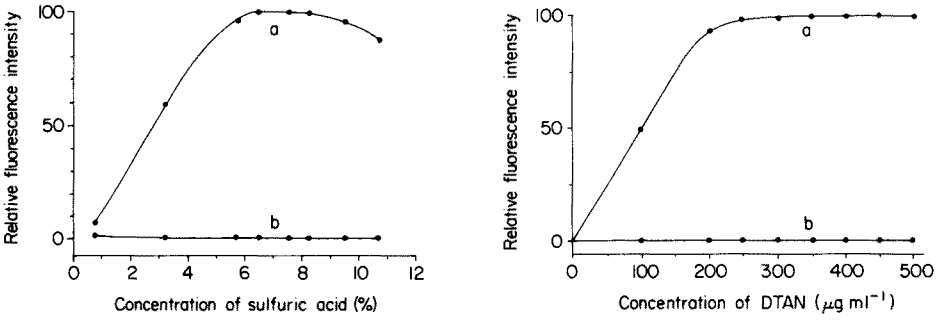


Fig. 2. Effect of the concentration of sulfuric acid in DTAN solution on the fluorescence development. Portions (1.0 ml) of 5 × 10^{-6} M *p*-hydroxybenzaldehyde solutions treated as recommended with DTAN dissolved in various sulfuric acid concentrations. (a) *p*-hydroxybenzaldehyde; (b) reagent blank.

Fig. 3. Effect of the concentration of DTAN on the fluorescence development. Portions (1.0 ml) of 5 × 10^{-6}M *p*-hydroxybenzaldehyde solutions were treated as recommended with various concentrations of DTAN. (a) *p*-Hydroxybenzaldehyde; (b) reagent blank.

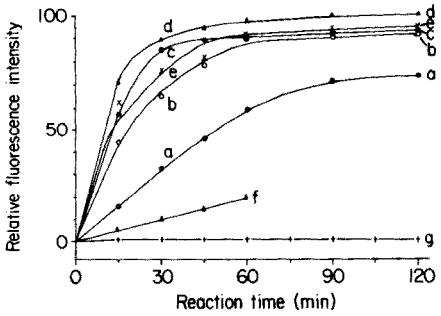


Fig. 4. Effect of reaction temperature and time, and presence of sodium sulfite and sodium phosphite on the fluorescence development. Portions (1.0 ml) of 5×10^{-6} M *p*-hydroxybenzaldehyde solutions were treated as recommended for various reaction times at the reaction temperatures (a) 0; (b) 23; (c) 37; (d) 50; (e) 70°C. Curve (f) is obtained by treating the aldehyde solutions as in the procedure without sodium sulfite and sodium phosphite; (g) is the reagent blank corresponding to a—f.

obtainable in a laboratory, and a reaction time of 30 min were selected to give an appropriate fluorescence intensity with economy of time.

Effect of β-mercaptoethanol and sulfuric acid on the fluorescence. Because the fluorescence develops, even at low ambient temperature, over long periods, the fluorescence reaction should be stopped immediately after the prescribed reaction time to obtain reproducible results. The addition of a 10% (v/v) β-mercaptoethanol solution stops the reaction abruptly and does not influence the fluorescence developed. A 5% solution can also be utilized but the blank fluorescence value is somewhat higher. The fluorescence intensity increases steadily as the concentration of sulfuric acid is increased from 3 to 6% (v/v) in the final reaction mixture, and almost maximal, constant fluorescence intensity is obtained when the mixture contains more than 6.5% of sulfuric acid. A concentration of ca. 7% in the final mixture, achieved by the addition of 1.0 ml of the β-mercaptoethanol solution in sulfuric acid, was selected.

When sodium hydroxide solution is added to give 2.7% of alkali in the mixture, the fluorescence, with excitation and emission maxima at 380 and 445 nm, respectively, becomes almost twice as intense as that in recommended acidic solution, but turbidity caused by the tri-n-butylphosphine gives a blank fluorescence which is 10 times higher than that in the final acidic mixture.

Stability of fluorescence and precision of procedure. The fluorescence developed under the prescribed conditions does not change on irradiation for 10 min at the excitation maximum of 372 nm; it is stable for 20 min in daylight, and for at least 3 days in the dark. The calibration graph is linear for aldehyde concentrations ranging from 2×10^{-8} to 2×10^{-5} M. The precision of the procedure was established by performing 17 analyses on 1×10^{-7} and 5×10^{-6} M aldehyde solutions. The coefficients of variation were 3.4 and 0.4%, respectively.

Fluorescence from other aldehydes

Many aromatic aldehydes fluoresce under the conditions recommended. The excitation and emission maxima, and the lower limits of determination are shown in Table 1. The excitation and emission maximum wavelengths are fairly characteristic of individual aldehydes. The proposed method is particularly sensitive for benzaldehyde derivatives with *p*-hydroxy, *o*- or *p*-methoxy, or *p*-methyl groups. However, some aromatic aldehydes do not fluoresce appreciably, even at a concentration of 1×10^{-5} M, under the recommended conditions; e.g. 2,5-dihydroxybenzaldehyde, *o*-vanillin, *m*-nitrobenzaldehyde, 2-hydroxy-1-naphthaldehyde, methylterephthalaldehyde and terephthalaldehydic acid. Arylaliphatic aldehydes (e.g. phenylacetaldehyde, cinnamaldehyde and *p*-dimethylaminocinnamaldehyde) and aliphatic aldehydes (e.g., formaldehyde, acetaldehyde, propionaldehyde, iso-valeraldehyde, n-butyraldehyde, crotonaldehyde and acrolein) do not fluoresce under the conditions recommended.

Reaction of substances other than aldehydes

Almost all other biologically important substances examined do not fluoresce at a concentration of 1×10^{-5} M, e.g. glycine, 17 different L-α-amino acids, glutathione, creatine, creatinine, histamine, tyramine, octopamine, dopamine, thiamine, citrulline, alloxan, allantoin, uric acid, bilirubin, urea, *N*,*N*-dimethylurea, acetone, cyclohexanone, 4-methylcyclohexanone, acetylacetone, acetophenone, resacetophenone, diacetyl, benzil, lactic acid, 3-hydroxybutyric acid, acetoacetic acid, pyruvic acid, oxaloacetic acid, 2-oxobutyric acid, 2-oxoglutaric acid, phenylpyruvic acid, homogentisic acid, inositol, D-xylose, D-glucose, D-fructose, D-galactose, D-mannose, D-maltose, D-lactose, D-raffinose, L-rhamnose, D-glucuronolactone, D-glucosaccharo-1,4-lactone, L-ascorbic acid, epiandrosterone, dehydroepiandrosterone, cortisone and cholesterol. *p*-Hydroxyphenylpyruvic acid gives a weak response with the reagent at a concentration of 5×10^{-6} M; the intensity is ca. double that of the reagent blank. Bovine serum albumin (fraction V) and soluble starch show no fluorescence at concentrations of 7 and 5 mg ml^{-1}, respectively.

The proposed method is very sensitive for *p*-hydroxybenzaldehyde, and may be applicable to the assay of dopamine β-hydroxylase in human and rat sera; the β-hydroxylation of tyramine gives octopamine which is easily converted to *p*-hydroxybenzaldehyde by periodate oxidation. Studies of the mechanism of the fluorescence reaction are in progress.

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AN ELECTROCHEMICAL STUDY OF COPPER(II) NITRATE AND PERCHLORATE IN *N,N*-DIMETHYLFORMAMIDE

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SUMMARY

Polarographic, cyclic voltammetric and controlled-potential coulometric studies of copper(II) nitrate and perchlorate in dimethylformamide are reported. Copper(II) in copper(II) perchlorate solutions is directly reduced in a 2e step to copper metal at platinum electrodes and to a copper amalgam at mercury electrodes. Copper(II) in the presence of nitrate forms a complex of composition $Cu(NO_3)_2$ in DMF; the dissociation constant, measured polarographically, is 9×10^{-5}. The copper(II) nitrate complex is electrochemically reduced in two steps consisting of a reversible dissociation of the complex followed by direct reduction of copper(II) ion to copper(0). The diffusion coefficients of copper(II) ion and the copper(II) nitrate complex are 4.91×10^{-6} cm^2 s^{-1} and 4.33×10^{-6} cm^2 s^{-1}, respectively.

Titrimetric data [1, 2] have shown that copper(II) chloride in *N,N*-dimethylformamide (DMF) is chemically reduced either to copper(I) or to a copper(I) complex. It is not clear whether copper(I) exists uncomplexed in DMF, or is stabilized either by chloride, as in water [3], or by the solvent. Chloride has been shown to form two copper(II) complexes in DMF [4]. In view of the relative importance of copper(II) as an oxidizing agent in DMF [1, 2, 5, 6], the present electrochemical study of copper(II) salts of two anions not expected to complex with copper(I) was undertaken.

EXPERIMENTAL

Chemicals

N,N-Dimethylformamide (Baker Analyzed Reagent) was stored under a dry argon atmosphere and was used without further purification. Reagent-grade copper(II) nitrate trihydrate (J. T. Baker) and copper(II) perchlorate hexahydrate (Alfa Products) were found, by atomic absorption spectrometry, to be 97.9 and 96.9% pure, respectively; all concentrations reported here were corrected appropriately. A 0.1 M solution of tetrabutylammonium perchlorate (TBAP; J. T. Baker) was used as the supporting electrolyte. Reagent-grade silver nitrate was used as received for the reference electrode

filling solution. All chemicals were stored in desiccators. Linde, high-purity argon was used to deaerate samples prior to electrochemical study and to protect the solvent and cell solutions from the atmosphere.

Apparatus

The model 174a Polarographic Analyzer (Princeton Applied Research) used was coupled either to an Omnigraphic 2000 x-y recorder (Houston Instrument) for voltammetry or to an Omniscribe strip-chart recorder (Houston Instrument) for controlled-potential coulometry. A Tektronix type 502 oscilloscope was used to record rapid cyclic voltammograms.

A silver—0.010 M silver nitrate reference electrode [2] and a platinum auxiliary electrode were used; the silver nitrate reference solution was prepared daily. All potentials reported in this paper are relative to this electrode. The working electrode was a platinum disc (0.1 mm diameter) for some studies of cyclic voltammetry, and a Metrohm hanging mercury drop electrode (h.m.d.e.; area = 2.2 mm^2) for the remaining studies. A dropping mercury electrode (d.m.e.) was used for polarographic studies, and either a mercury pool or a platinum foil for controlled-potential coulometry. All polarographic diffusion current measurements were made at the top of the undamped recorder traces.

The platinum disc electrode used for cyclic voltammetry was pretreated before each set of runs by dipping in concentrated nitric acid, rinsing, and then polishing the platinum with laboratory tissues. The water-jacketed, three-compartment cell was maintained at 25.0°C unless otherwise indicated. The two outer compartments in the cell were separated from the center compartment by medium-porosity glass frits.

RESULTS AND DISCUSSION

Copper(II) perchlorate

Copper(II) perchlorate in DMF had a single cathodic wave at a half-wave potential ($E_{1/2}$) of −0.383 ± 0.011 V (Fig. 1) for 13 solutions of varying concentration between 1 and 8 mM. (All uncertainties listed in this paper are expressed as standard deviations.) The random variation in $E_{1/2}$ with concentration can be attributed to slight daily variations in the reference electrode potential and in other experimental parameters. The temperature coefficient of the half-wave potential of 2.2 mM copper(II) perchlorate, measured at seven temperatures from 10 to 40°C, was found to be +0.68 mV/degree. This variation is within the expected range for a reversible electrode reaction [7a]. Plots of E as a function of log $[i/(i_d - i)]$ were obtained on six samples. The average slope of the resulting straight lines was −0.036 ± 0.006 V/decade. This value is considerably closer to the theoretical slope for a reversible two-electron transfer (−0.030 V/decade) than to the theoretical slope for a reversible one-electron transfer (−0.059 V/decade).

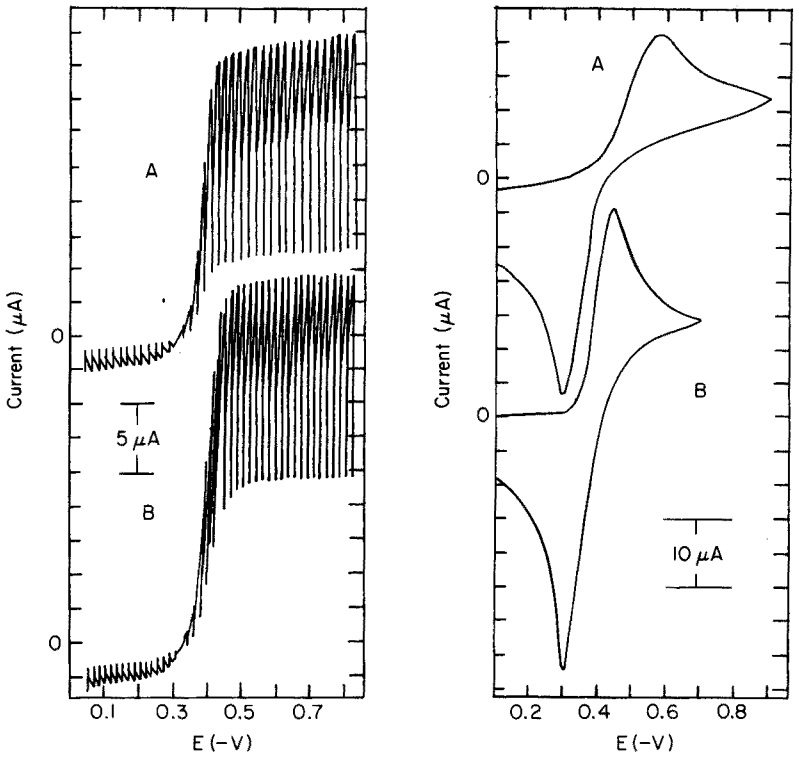

Fig. 1. Polarograms of DMF solutions. (A) 4.1 mM copper(II) perchlorate; (B) 5.1 mM copper(II) nitrate.

Fig. 2. Cyclic voltammograms obtained at the h.m.d.e. (A) 2.9 mM copper(II) nitrate at 0.1 V s^{-1}; (B) 5.1 mM copper(II) perchlorate at 0.05 V s^{-1}.

The diffusion current (i_d) of the polarographic wave increased linearly with the square root of the corrected mercury column height (h_{corr}), and $i_d/h_{corr}^{1/2}$ decreased slightly with h_{corr}. These results are expected for an electroactive species which obeys the Ilkovic and Koutecky equations [7b], and indicate diffusion control of the height of the polarographic wave. The relative temperature coefficient of the diffusion current measured at five temperatures between 15° and 35°C was +0.7%/degree, which is sufficiently close to the theoretical value of about +1.3%/degree (7c) to indicate the absence of slow chemical steps in the electroreduction.

Exhaustive controlled-potential coulometry of four copper(II) perchlorate solutions at −0.60 V at a mercury pool electrode yielded a calculated n of 1.99 ± 0.03 mol of electrons transferred for each mole of copper(II) perchlorate. Exhaustive electrolysis was also carried out on four copper(II) perchlorate solutions at −0.80 V at a platinum foil electrode. In each case, the foil was plated with copper during the electrolysis. The calculated n for these four electrolyses was 1.99 ± 0.05. Polarograms of the solutions after

each exhaustive electrolysis at both the mercury and platinum working electrodes contained no waves between 0 and −3 V.

The polarographic diffusion current increased linearly with increasing concentration (C) from 0.2 to 8.0 mM. From the Ilkovic equation, $i_d = (708nD^{1/2}m^{2/3}t^{1/6})C$, and the slope of the $i_d - C$ plot, with $n = 2$ and the measured $m^{2/3}t^{1/6}$ value of 1.59 $mg^{2/3}\ s^{-1/2}$, the diffusion coefficient for copper(II) perchlorate was calculated to be $4.91 \times 10^{-6}\ cm^2\ s^{-1}$.

Cyclic voltammograms recorded at the h.m.d.e. at scan rates between 0.020 and 5 V s^{-1} contained one cathodic peak between −0.4 and −0.5 V and one anodic peak between −0.26 and −0.35 V (Fig. 2). The anodic peak height was always greater than the cathodic peak height, and increased in height as the switching potential became more negative at constant scan rate and with decreased scan rate at a fixed switching potential. Both of these observations indicate deposition on the electrode during the cathodic scan followed by stripping during the anodic scan. Longer scan times resulted in more time for deposition and therefore a larger anodic peak. The cathodic peak current increased linearly with square root of scan rate to about 0.5 V s^{-1}. At higher scan rates a small overlapping prepeak was observed at about −0.3 V, which made current measurements unreliable. As scan rate increased, the separation between the cathodic and anodic peaks also increased. The separation between the peaks was sufficient to classify the electrochemical reaction as either quasi-reversible or irreversible depending on the scan rate [8].

At the platinum disc, cyclic voltammetry revealed one cathodic peak between −0.53 and −0.65 V and a prepeak at about −0.5 V (Fig. 3). At scan rates above 0.5 V s^{-1}, the two peaks appeared to merge into a single, relatively broad peak. Upon scan reversal, a single anodic peak was observed at about −0.3 V. The anodic peak behaved in a manner analogous to its behavior at the h.m.d.e. and therefore can be attributed to anodic stripping. The cathodic peak current increased linearly with square root of scan rate to about 0.1 V s^{-1}. At higher scan rates current measurements were unreliable because of some overlapping by the broad platinum oxide peak at about −0.3 V. As scan rate increased, the separation between the cathodic and anodic peaks increased. In all cases, however, the separation between the cathodic and anodic peaks was large enough to classify the reduction as irreversible on platinum.

The available evidence indicates the single-step, two-electron reduction of copper(II) perchlorate, on both mercury and platinum, to copper(0) $Cu^{2+} + 2e \rightarrow Cu$. The reduction can be qualitatively described as reversible at the d.m.e. under polarographic conditions and quasi-reversible or irreversible at both mercury and platinum electrodes under the cyclic voltammetric conditions of more rapid scan rate.

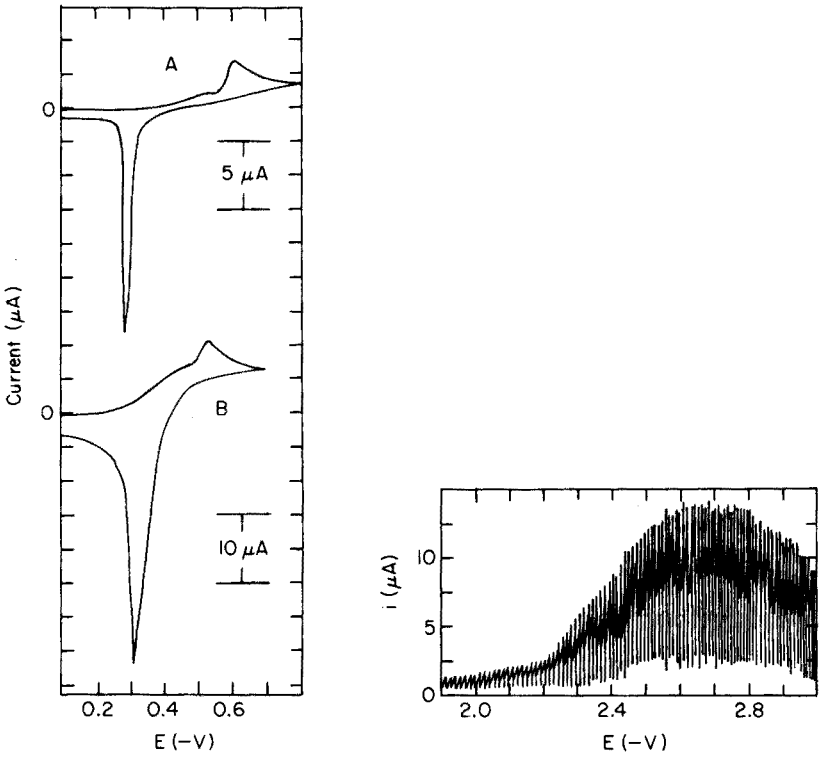

Fig. 3. Cyclic voltammograms obtained at the platinum disc. (A) 2.8 mM copper(II) perchlorate at 0.02 V s^{-1}; (B) 5.3 mM copper(II) nitrate at 0.05 V s^{-1}.

Fig. 4. A polarogram taken after exhaustive controlled potential coulometry at —0.6 V on a mercury pool of 6.4 mM copper(II) nitrate.

Copper(II) nitrate

Copper(II) nitrate in DMF had a single, cathodic, polarographic wave (Fig. 1) at a half-wave potential of —0.396 ± 0.010 V for 13 solutions of varying concentration between 0.2 and 9.3 mM. The variation in $E_{1/2}$ with concentration was apparently random. The temperature coefficient of the half-wave potential of 2.9 mM copper(II) nitrate, measured at five temperatures between 20 and 40°C, was +0.83 mV/degree, which is within the range expected for reversible waves [7a]. Plots of E as a function of log $[i/(i_d - i)]$ were made for seven samples of varying concentration. The average slope was —0.035 ± 0.005 V/decade. As was the case for copper(II) perchlorate, this value is only slightly more negative than the theoretical slope for a reversible, two-electron wave (—0.030 V/decade). The diffusion current increased linearly with the square root of the corrected mercury column height and $i_d/h_{corr}^{1/2}$ decreased slightly with h_{corr}, indicating diffusion control of the wave [7b]. The relative temperature coefficient, measured at five temperatures between 20 and 40°C, was +0.9%/degree; this relatively

low value indicates the absence of slow chemical reactions within the diffusion layer [7c].

Exhaustive controlled-potential coulometry of six copper(II) nitrate solutions at —0.60 V at a mercury pool electrode and of four solutions at —0.70 V at a platinum foil electrode yielded calculated values of n of 2.0 ± 0.1. As with copper(II) perchlorate, the diffusion current of copper(II) nitrate increased linearly with concentration, allowing the use of Ilkovic equation. The diffusion coefficient of copper(II) nitrate was calculated to be 4.33×10^{-6} cm^2 s^{-1}.

After each exhaustive electrolysis, a polarogram of the resultant solution revealed the disappearance of the original cathodic wave at —0.4 V. In some cases, a new cathodic wave or possibly two new overlapping waves appeared between —2.2 and —2.5 V (Fig. 4); where this new wave was observed, the relative height of the wave after each electrolysis was not constant with respect to the height of the original cathodic wave.

Apparently the new wave was not due to a copper species, because the current consumed during each electrolysis indicated the removal of all copper from solution as either a copper amalgam or a copper plate; in fact copper plating was observed on the platinum foil electrode during each electrolysis. Polarograms of the background electrolyte solution (0.1 M TBAP). occasionally revealed a similar wave at the same potential. Consequently, it appears likely that the new wave was due to some product in the electrolyte-solvent system, and was not related to the electrochemical reaction of copper(II) nitrate. Conditions were favorable for a chemical reaction during each coulometric study, because rapid stirring was typically maintained for about 1 h.

The slightly more negative $E_{1/2}$ of the copper(II) nitrate wave (—0.40 V) compared to that of the copper(II) perchlorate wave (—0.38 V), and the smaller diffusion coefficient of copper(II) nitrate (4.33×10^{-6} cm^2 s^{-1}) compared to that of copper(II) perchlorate (4.91×10^{-6} cm^2 s^{-1}) suggested the possibility of copper(II) complex formation with nitrate. This possibility was checked polarographically. Six solutions of 1.07 mM copper(II) perchlorate were prepared in DMF containing ammonium nitrate at concentrations varying from 0.05 M to 0.1 M; the total ionic strength was maintained at 0.1 M by the addition of TBAP as required. A plot of $E_{1/2}$ as a function of log [NO_3^-] for these solutions was a straight line with a slope of —0.059 V/decade, which indicates complexation. For a general reaction of the form $MX_p^{(n-pb)+} + ne + Hg = M(Hg) + pX^{-b}$, the slope of the $E_{1/2}$—log [X^{-b}] plot is —0.05915 p/n at 25° [7d]. In this particular case, $n = 2$, and consequently p, the number of ligands in the complex, must also equal 2, i.e., the species in solution is $Cu(NO_3)_2$.

The dissociation constant of the complex was estimated from the difference in $E_{1/2}$ between 1.07 mM copper(II) perchlorate and 1.07 mM copper(II) perchlorate in 0.1 M nitrate by use of equation (1) [7d]

$$(E_{1/2})_c - (E_{1/2})_s = 0.0296(\log K_D - \log f_s/f_c - \log D_c^{1/2}/D_s^{1/2} - 2\log 0.1 f_{NO_3^-}) \quad (1)$$

where subscripts c refer to the complex and subscripts s refer to the copper(II) species in the absence of nitrate; f is the activity coefficient and K_D the dissociation constant. Log f_s/f_c can be estimated [7d] from the extended Debye—Huckel equation and $f_{NO_3^-}$ from the Debye—Huckel equation. When these values and the diffusion coefficients calculated earlier were substituted in eqn. (1), the dissociation constant of the copper(II) nitrate complex was found to be 9×10^{-5}.

Cyclic voltammetric studies were conducted (Figs. 2 and 3) at both the h.m.d.e. and platinum disc at scan rates of 0.02—5 V s^{-1}. At the mercury electrode, a single cathodic peak was observed between —0.4 and —0.6 V; scan reversal showed a single anodic peak between —0.30 and —0.38 V. At the platinum disc, the cathodic peak occurred between —0.5 and —0.7 V, and the anodic peak was found between —0.21 and —0.31 V. A cathodic prepeak was observed on platinum at about —0.45 V. In each case the anodic peak had those characteristics described earlier of a stripping peak. Application of the diagnostic criteria of Nicholson and Shain [9] to the cathodic peak indicated that the reaction at the electrode consisted of a reversible preceding chemical reaction followed by an irreversible electron transfer. The separation between the cathodic and anodic peaks was larger, at a fixed scan rate, for the platinum electrode than for the mercury electrode, indicating that the electrode reaction was more irreversible on platinum.

From the available evidence, the electrochemical reaction mechanism at either platinum or mercury electrodes consists of dissociation of the copper(II) nitrate complex ($K_D = 9 \times 10^{-5}$) followed by reduction of copper(II) to copper(0). Although the overall reduction is nearly reversible under polarographic conditions, as indicated by slopes of plots of E as a function of $\log[i/(i_d - i)]$, at the faster scan rates employed for cyclic voltammetry, the reduction becomes irreversible.

These studies show that in the absence of ligands such as chloride, which form stable complexes, copper(I) does not exist in DMF solutions.

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THE EFFECT OF SURFACTANTS ON THE CONCENTRATION OF HEAVY METALS FROM NATURAL WATERS ON CHELEX-100 RESIN

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SUMMARY

Trace amounts of zinc, cadmium, copper, nickel, manganese, cobalt and lead can be separated from natural waters on Chelex-100 resin (50-100 mesh) in the presence of cationic, anionic and non-ionic detergents, washing powder and sodium tripolyphosphate at concentrations as high as 100 mg l^{-1}. Metal recoveries are better than 92% but are poor in the presence of soap or the potential detergent additive, nitrilotriacetic acid. Although strong adsorption of cationic, and to a lesser extent, anionic and non-ionic detergents, occurs on the resin surface, low recoveries can be attributed to incomplete metal elution rather than to blockage of adsorption sites. Experiments with radiotracers show that the total metal present in natural waters is not adsorbed by Chelex-100 unless metal ions are first released from colloids or strong complexes.

The chelating resin Chelex-100, a purified form of Dowex A1 resin, has been increasingly used in recent years for the separation and preconcentration of trace metals from natural waters [1—10]. It is particularly suited to this application because optimum metal removal occurs in the pH range of natural waters, pH 6—8, where distribution coefficients of the order of 10^5 have been measured [11].

In most instances, the resin has been used in the H^+-form, although the effluent pH under these conditions may be as low as 2.8. Florence and Batley [12] showed that the H^+-form did not completely remove labile zinc, cadmium, lead and copper from sea water, until the passage of more than 500 ml of sample had increased the pH of the effluent to 6.5. Treatment of the column with sodium acetate before use raised the effluent pH to 7.1. The ammonium or calcium forms also have been used to circumvent this problem [3, 13, 14].

Several papers have reported distribution data for selected metal ions on Chelex-100 as a function of pH [14, 15]. Batch equilibration studies reported by Dow Chemical Company [1] show complete removal of Co, Ni and Cu at

pH 4, while similar results have been obtained for Zn, Cd and Mn [11], and for Fe(III) and Pb [7]. Exchange kinetics are slow at low pH values where the imino nitrogen of the resin functional group is protonated [16]; however, at the pH of natural waters, exchange is rapid, and efficient column operation is observed in a 1.5-ml column with flow rates of 1.5—2.5 ml min^{-1}.

For the adsorption of trace metals from sea water, Riley and Taylor [2] recommended a pH of 7.6, with a flow rate of 5 ml min^{-1} through 100-200 mesh resin. Recovery of Fe and Mn was incomplete, the latter requiring a pH of 9.0 for complete recovery.

Biechler [17] examined metal removal from industrial waste waters on Dowex-A1 (50-100 mesh) in the sodium form. For solutions adjusted to pH 5.2, complete column retention of Cd, Zn, Cu, Ni and Pb was noted. Retention of iron(III) and chromium(III) was incomplete. No attempt was made to purify the commercial resin or to control resin pH.

This paper describes the use of Chelex-100 for the preconcentration of metals from natural and polluted non-saline waters and, in particular, looks at the effect of some organic surface-active pollutants on the efficiency of metal removal. These pollutants include cationic, anionic and non-ionic detergents, formulated detergents, detergent additives and a soap, which are all likely domestic and industrial discharges into river systems.

EXPERIMENTAL

Apparatus and reagents

The exchange columns were 13-cm long, 0.8-cm bore glass tubes with a 1.2-mm Interflon stopcock and a 1.0-cm socket at the top to receive a 60-ml glass reservoir.

Chelex-100 resin (Bio-Rad Laboratories, Richmond, California, USA), 50-100 mesh, was supplied in the sodium form.

Standard metal solutions were prepared from analytical-grade reagents. Acetate buffer solution was prepared by dissolving 238 g of sodium acetate trihydrate in 500 ml of water, adding 102 ml of glacial acetic acid, and diluting to 1 l.

Distilled water, prepared by distilling demineralized water from permanganate, was stored in a polythene tank.

Recommended procedures

Preparation of column. Fill the ion-exchange column with resin to a height of 7.5 cm. Pass through the column 20 ml of water containing 1.0 ml of 10% sodium hydroxide, then pass 40 ml of distilled water, followed by 20 ml of water containing 1.8 ml of acetate buffer. Wash the column with water (2 × 20 ml). The pH of the last 10 ml of effluent (called in this paper "the pH of the column") should be between 6.5 and 6.7. If the pH is too low, it can be increased by successive washings of the column with water. Use the column immediately.

The column can be converted to the sodium form and stored after elution of metals with acids, by washing with 30 ml of water and passing through 10 ml of 10% sodium hydroxide (high purity) solution, followed by 50 ml of water. A column may be re-used up to eight times.

Sample preparation and storage. After sampling, filter the water through a 0.45-μm membrane filter. It is preferable to analyse the filtered sample immediately. If this is inconvenient, the sample may be stored in acid-washed polythene bottles after the addition of 2.5 ml of 15 M nitric acid per litre of filtrate.

Analytical method for natural waters. Adjust the pH of the column to 6.5—6.7. If the pH of the sample is greater than pH 7.5, adjust a 250-ml sample to pH 6.6 with 0.05 M nitric acid. For acidic samples, or when standard addition is used to determine recoveries, add 5 mg of sodium tripolyphosphate to a 250-ml sample to prevent possible precipitation of metal hydroxides in subsequent pH adjustment. When larger sample aliquots are required, increase the amount of sodium tripolyphosphate accordingly. Adjust the pH to 6.5–6.7 with 0.05 M sodium hydroxide, in a carefully stirred solution.

Pass the sample through the column at a rate not exceeding 3 ml min^{-1}. Rinse the beaker with 10 ml of distilled water and add to the column.

Elute the metals with 25 ml of 2 M nitric acid, followed by 5 ml of water, 10 ml of 2 M hydrochloric acid and 5 ml of water. Reduce the volume of the eluate to 20 ml by boiling. Cool, transfer to a 25-ml volumetric flask and dilute to volume with distilled water. Determine the metals by atomic absorption spectrometry (a.a.s.). Determine the column blank and subtract from the results.

RESULTS AND DISCUSSION

Column preparation

In the preparation of the resin column, a careful washing procedure must be followed to adjust the resin pH to the optimum value for metal chelation. On the basis of published distribution data and values for the apparent acid dissociation constants of iminodiacetate functional groups, the resin is in the monosodium zwitterion form at the optimum pH of 7.4. As supplied, the resin is in the disodium form and usually contains considerable free OH^- in the pore structure. Ineffective washing will result in precipitation of hydroxides of the readily hydrolysed metals, Cr, Mn, and Fe, which will block the column.

Column preparation is best carried out by batch equilibration of resin with an excess of acetate buffer of the desired pH. For continuous column operation, with acid elution of metals, the column is best regenerated by washing first with high-purity sodium hydroxide solution, and then with distilled water and acetate buffer.

For most natural waters, it was unnecessary to adjust the sample pH before addition to the column, but the pH of acidic samples was first adjusted to 6.5—6.7 with dilute sodium hydroxide. To avoid possible precipitation of iron and manganese hydroxides, sodium tripolyphosphate (20 mg l^{-1}) was added immediately before pH adjustment. It was shown that this addition did not alter metal recoveries. Storage of unacidified samples containing sodium tripolyphosphate for up to 14 days resulted in slow hydrolysis of some metal polyphosphate complexes with the appearance of a dark colloidal precipitate, possibly hydrated manganese(IV) oxide. Thus although sodium tripolyphosphate hinders the precipitation of metal hydroxides, it cannot replace acid for stabilizing dilute metal solutions for long periods.

The effluent pH values for a correctly washed column should lie in the range 6.5—7.5.

Although it is common practice to elute metals from the column with 2 M nitric acid [2, 6], this acid alone did not completely elute manganese despite the presence of sodium tripolyphosphate and careful pH adjustment. Washing with distilled water followed by elution with 2 M hydrochloric acid assisted greatly in the removal of manganese from the column. Hydrochloric acid alone resulted in poor recoveries of cadmium, zinc and lead. Elution with both acids is therefore recommended.

Effects of surfactants

The recoveries of Zn, Cd, Cu, Ni, Mn, Co and Pb from distilled water solutions were examined in the presence of the following surfactants:

(1) cationic detergent — cetyldimethylbenzylammonium chloride (100% active material);

(2) anionic detergent — linear alkylated sulphonate (LAS) (100% active material);

(3) non-ionic detergent — nonyl phenol ethoxylate (100% active material);

(4) industrial LAS-type detergent — 74% LAS-type detergent and 6% sodium tripolyphosphate;

(5) formulated detergent — sulphonated alkylbenzyl type, neutralized with coconut oil ethanolamine (13% active material);

(6) a washing powder — a formulated product containing 15% LAS-type detergent and 25% sodium tripolyphosphate;

(7) a soap.

In addition, sodium pyrophosphate, sodium tripolyphosphate and its potential substitute, nitrilotriacetic acid (NTA), were examined.

The concentrations of surfactants shown in this paper are the concentrations of the product supplied by the manufacturer, which is not necessarily equal to the active material present. Commercially prepared products can contain, in addition to the detergents, foam stabilizers, emulsifying agents and grease-cutting compounds such as polyphosphates; therefore, it would be incorrect to attribute interferences to the surfactants only. In order to exaggerate any possible interferences, surfactants were studied at much higher concentrations than would be expected in polluted waterways.

The results (Table 1) show that excellent (generally >95%) metal recoveries were obtained in most cases. Notable exceptions were recoveries in the presence of soap and nitrilotriacetic acid (NTA). The latter can compete successfully with the iminodiacetate groups of the resin, thus reducing resin efficiency. The equilibrium lies in favour of the resin and retention is facilitated by reducing the solution flow rate. For example, the recovery of zinc (0.5 mg l^{-1}) in the presence of NTA (100 mg l^{-1}) was increased from 32% to 50% by reducing the flow rate to 2 ml min^{-1}. Similar results have been reported by Stolzberg and Rosin [18].

Samples containing soap, after adjustment to pH 6.4, formed a precipitate of fatty acids which eventually collected on the top of the resin column, fouling it. Recoveries of manganese and cobalt in the presence of 20 mg l^{-1} soap were low.

As might be expected, surfactants are themselves strongly adsorbed by Chelex-100. Table 2 shows the removal by the column of standard aliquots of cationic, anionic and non-ionic surfactants. Cationic and non-ionic surfactants were determined in the effluent by u.v. absorbance measurements, while the anionic surfactant was measured by the standard methylene blue reactive substances method. The maximum adsorption of cationic detergent corresponded to 13% of the total exchange capacity of the resin. Since the cetyldimethylbenzylammonium chloride molecules are too large to enter the

TABLE 1

Effect of surfactants on the percentage recovery of heavy metals by Chelex-100 (The concentrations of metals added were 0.5 mg l^{-1} for Zn and Cd, 1.0 mg l^{-1} for Cu, Ni, Mn and Co, and 3.0 mg l^{-1} for Pb)

Surfactant	Concn. (mg l^{-1})	Metal recovery (%)[a]						
		Zn	Cd	Cu	Ni	Mn	Co	Pb
No additions	—	100	100	100	100	100	100	100
Cationic detergent	100	98	95	98	100	97	100	94
Anionic detergent	100	94	95	98	97	97	98	95
Anionic detergent	10	98	98	—	—	99	—	97
Non-ionic detergent	100	95	96	98	97	99	96	97
Industrial detergent (LAS-type)	100	95	96	98	97	99	96	97
Formulated detergent	500	95	95	92	99	97	95	95
Washing powder	500	94	95	93	98	90	99	98
Sodium pyrophosphate	100	98	95	97	99	97	97	97
Sodium tripolyphosphate	100	96	95	100	100	97	97	97
Sodium tripolyphosphate	20	96	96	—	—	97	—	—
Sodium tripolyphosphate	10	98	97	—	—	97	—	—
Soap	20	—	—	—	—	78	80	—
NTA	100	32	58	90	4	91	50	46
NTA	10	37	70	95	4	91	55	48

[a]From 200-ml samples.

TABLE 2

Adsorption of detergents on Chelex-100

Detergent concn. (mg l^{-1})	% Adsorbed[a]		
	Cationic	Anionic	Non-ionic
20	100 (4)	35 (1.4)	82 (3.3)
100	100 (20)	20 (4)	80 (16)
500	100 (100)	14 (14)	42 (42)
1000	88 (176)	10 (20)	26 (52)
2500	39 (195)	4 (20)	11 (55)
5000	20 (200)		5.5 (55)

[a]Figures in brackets are mg adsorbed from 200-ml samples on 5.5 ml of Chelex-100 resin.

TABLE 3

Distribution of manganese, zinc, cobalt and cadmium determined by tracer addition method after separation on Chelex-100 (50-100 mesh) resin from 200-ml samples

Metal (μg)	Surfactant (mg l^{-1})		Distribution (%)		
			In effluent	Eluted	Retained on column
Mn (50)	Washing powder	500	1	91	8
	NTA	100	12	88	0
	Sodium tripolyphosphate	100	1	97	2
	Sodium tripolyphosphate	20	0	100	0
	Anionic detergent	100	0	98	2
	Anionic detergent	20	0	100	0
Zn (20)	Washing powder	500	1	93	6
	Sodium tripolyphosphate	100	0	97	3
	Sodium tripolyphosphate	20	0	100	0
	Anionic detergent	100	3.5	94	2.5
	Anionic detergent	20	2	98	0
Co (40)	Washing powder	500	1	98	1
	Sodium tripolyphosphate	100	2	97	1
	Anionic detergent	100	0.5	98.5	1
	Non-ionic detergent	100	0	96	4
Cd (20)	Washing powder	500	3	95	2
	Sodium tripolyphosphate	100	1	96	3
	Anionic detergent	100	0.5	94	5.5
	Non-ionic detergent	100	1	93	6

resin micropore network, this figure must represent adsorption on surface sites. Only 2 and 3% of the total exchange capacity was used for anionic and non-ionic detergents, respectively. A resin saturated with cationic detergent still retained 99.8% of the added zinc.

The efficiency of Chelex-100 in concentrating metals in the presence of surfactants and the subsequent effectiveness of the eluant were studied with radiotracers. The tracer solutions, ^{54}Mn, ^{65}Zn, ^{60}Co and ^{115}Cd, were equilibrated with the corresponding standard metal solutions, surfactant was added and the recommended procedure followed. Effluent and eluate fractions were collected and the distribution of the metals determined by gross γ-counting in a well-crystal γ-counter. The results are shown in Table 3.

Blocking of adsorption sites by surfactants did not occur, and metal adsorption was very good in the presence of the surfactants studied. Low metal recoveries could, in general, be attributed not to initial blockage of adsorption sites but to the incomplete removal of metal from the column with the acid eluant. This could result from metal being trapped within the resin structure as the volume of the resin decreased (normally two-fold) on elution with acid. During this step, detergents, particularly the anionic and non-ionic species, could hinder elution by partially blocking the resin pores.

In the presence of NTA, incomplete metal tracer adsorption was observed. Similar results were obtained in the presence of humic acids.

Effects of mesh size

Chelex-100 has been used in both 50-100 and 100-200 mesh ranges for metal preconcentrations, and it is generally accepted that exchange is more efficient with the smaller beads. However, it was found that, while the smaller mesh is desirable where metal removal from the effluent is being measured, the larger size is preferable if the exchanged metals are to be recovered from the column. Elution of metals with acid from 100-200 mesh resin is particularly inefficient (Table 4) and, in fact, for complete metal recoveries, only small concentration factors can be achieved unless large sample volumes are used.

At least 25 ml of dilute nitric acid was required to elute metals from a 50-100 mesh resin. In agreement with Satake et al. [6], more than 100 ml of acid was needed for 100-200 mesh resin.

Examination of natural waters

The recommended procedure was used to determine the concentration of heavy metals in non-saline water samples from 12 creeks in the Sydney (NSW) area (Table 5). The creek samples included industrial waste waters (A), those receiving effluent from activated sludge treatment plants (B, C, D), creeks from unsewered areas liable to contain effluent from domestic absorption pits (E–K), and unpolluted waters (L). Anionic surfactants present were estimated with the methylene blue reagent. The lowest metal recovery was 86% and, in general, recoveries based on standard additions exceeded 90%.

Sodium tripolyphosphate (20 mg l^{-1}) was added to assist in complexing iron and manganese, which might otherwise precipitate when the pH is

TABLE 4

Effect of resin mesh size on recovery of heavy metals on Chelex-100
(The concentrations of metals added were 0.5 mg l^{-1} for Zn and 1.0 mg l^{-1} for Cu and Co)

Volume of 1 M HNO_3 eluant (ml)	Surfactant (100 mg l^{-1})	Recovery (%)					
		Cu		Zn		Co	
		50—100	100—200	50—100	100—200	50—100	100
0—50	—	100	92	99	87	100	89
50—100	—	—	8	1	12	—	10
100—150	—	—	—	—	1	—	1
0—50	Anionic	98	83	94	60	98	75
50—100	detergent	2	6	5	15	2	8
100—150		—	5	1	12	—	10
0—50	Sodium	100	85	96	67	100	74
50—100	tripolyphosphate	—	7	4	13	—	14
100—150		—	6	—	14	—	10

TABLE 5

Results for metals and methylene blue reactive substances (MBRS) in creeks from the Sydney area

Sample location	MBRS (mg l^{-1})	Zn	Cd	Cu	Ni	Mn	Co	Pb
		($\mu g\ l^{-1}$)[a]						
Alexandra Canal, Mascot (A)	0.11	200 (96)	2.7 (100)	52 (94)	15 (92)	103 (84)	8 (94)	31 (94)
Breakfast Creek, Quakers Hill (B)	0.58	200 (95)	<0.03 (97)	9 (88)	22 (93)	150 (91)	<2 (97)	<6 (94)
South Creek, St Marys (C)	0.61	1.4 (98)	<0.3 (95)	2 (86)	15 (97)	250 (90)	<2 (99)	<6 (94)
Cattai Creek, Kellyville (D)	0.38	2.9 (98)	<0.3 (98)	6 (94)	6 (96)	82 (89)	<2 (99)	<6 (93)
Lane Cove River, West Pymble (E)	0.07	16 (90)	<0.3 (93)	3 (97)	<3 (97)	41 (89)	<2 (97)	<6 (93)
French's Creek, Belrose (F)	0.90	15 (95)	<0.3 (97)	2 (97)	<3 (92)	50 (93)	<2 (97)	<6 (90)
Middle Harbour Creek, St Ives (G)	0.86	15 (97)	<0.3 (97)	3 (100)	<3 (93)	31 (90)	<2 (97)	<6 (91)
Oxford Creek, Oxford Falls (H)	0.20	26 (95)	<0.3 (98)	1 (98)	<3 (92)	35 (89)	<2 (97)	<6 (96)
Carrol Creek, Forestville (I)	0.03	15 (94)	<0.3 (95)	1 (98)	<3 (92)	11 (92)	<2 (96)	<6 (90)
Ku-ring-gai Creek, St Ives (J)	0.08	5.4 (100)	<0.3 (99)	7 (100)	<3 (91)	30 (90)	<2 (97)	<6 (95)
Cowan Creek, N. Turramurra (K)	0.19	1.6 (100)	<0.3 (99)	2 (96)	<3 (92)	30 (90)	<2 (97)	<6 (89)
Neverfail Creek, Terry Hills (L)	0.04	1.3 (98)	<0.3 (97)	3 (97)	<3 (92)	8.5 (92)	<2 (100)	<6 (94)

[a]Figures in brackets are % recovery of standard addition.

adjusted with alkali. To ascertain whether this addition had any undesirable effect, metal concentrations were determined in five of the creeks studied, with and without the addition of sodium tripolyphosphate. No variation in metal recoveries was observed.

With some of the natural water samples, zinc and manganese were present to such an extent that they could be determined by a.a.s. by direct aspiration into the flame. In these cases, the results were in excellent agreement with those observed after preconcentration on Chelex-100.

To gain insight into the fraction of total metal determined in natural waters after concentration by Chelex-100, small quantities of manganese-54, zinc-65, cobalt-60 and cadmium-115 tracers were mixed with four different filtered natural water samples. These were allowed to stand for up to seven days before the mixtures were put through the resin column. The proportion of metal tracer adsorbed by the resin decreased with standing time (Table 6). It was not possible to extend this study to longer standing times because of extensive adsorption of tracers (up to 10%) onto the glass walls of the equilibration vessels. For this reason, the values quoted in Table 6 must be regarded as minimum values, especially after 7 days' standing. Equilibrium may well be established after this period, because the decrease in adsorption by the resin after standing for 3—7 days is not great.

Because up to 28% of tracer was not recoverable by the resin after storage for seven days, the metal concentrations quoted in Table 5 must not

TABLE 6

Adsorption on Chelex-100 of manganese-54, zinc-65, cobalt-60, and cadmium-115 tracers after equilibration with natural water samples

Water sample	Metal	% of tracer adsorbed on resin after:		
		1 day	3 days	7 days
French's Creek, Belrose	^{54}Mn	100	99.5	99.5
	^{65}Zn	91	80	75
	^{60}Co	90	78	74
	^{115}Cd	80	75	72
Breakfast Creek, Quakers Hill	^{54}Mn	100	98	95
	^{65}Zn	94	91	88
	^{60}Co	91	82	80
	^{115}Cd	93	92	90
Eastern Creek, Doonside	^{54}Mn	99.5	86	85
	^{65}Zn	96	92	88
	^{60}Co	87	83	82
	^{115}Cd	91	90	90
Heathcote Creek, Heathcote	^{54}Mn	100	99.8	99.5
	^{65}Zn	100	99.5	99
	^{60}Co	100	99.5	99
	^{115}Cd	100	97	95

be regarded as total metal concentrations. Moreover, any metal present either as strong complexes which do not (or only slowly) exchange with the iminodiacetate resin groups, or as metal associated with colloidal particles, would not be retained by the chelating resin. Colloidal particles cannot penetrate the pore network of Chelex-100 which has an average pore diameter of only 6 nm

To ensure that total metal concentrations of natural waters are being measured, destruction of organic matter by u.v. irradiation and dissolution of colloids by acid treatment is advisable before any resin preconcentration. Irradiation for 4 h with a 550-W lamp of 100-ml samples, to which were added 0.7 ml of 15 M nitric acid and 0.05 ml of 30% hydrogen peroxide, has been shown to release bound metal completely [19]. When either this procedure or a total acid digestion was carried out on several water samples, tracer removal by the resin was complete and the recovered metal concentrations increased accordingly.

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N-SUBSTITUTED PHENOTHIAZINES AS INDICATORS IN TITRATIONS WITH CHLORAMINE-T

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SUMMARY

Trifluoperazine dihydrochloride, butaperazine dimaleate, promethazine hydrochloride, diethazine hydrochloride, prochlorperazine maleate and chlorpromazine hydrochloride have been studied as redox indicators in titrations of hydroquinone, metol and ascorbic acid with chloramine-T. The end-points obtained are sharper than with conventional indicators. The molar absorptivities of the oxidized indicators are reported. Simple but accurate methods for the determination of hydroquinone and metol are described.

In titrations with chloramine-T, the commonly used indicator, starch, has the disadvantage that the substance to be determined must be a stronger reducing agent than the iodide present, otherwise iodine would separate on adding chloramine-T. Some organic dyestuffs used as irreversible indicators are destroyed by the first drop in excess of the titrant at the equivalence point [1]. Only seven reversible indicators have been proposed, five in the titration of arsenic(III) [1] and two in the titration of ascorbic acid [2, 3].

The present paper describes a study of *N*-substituted phenothiazines as redox indicators and the development of new methods for the determination of ascorbic acid, hydroquinone and metol with trifluoperazine dihydrochloride (TFP), butaperazine dimaleate (BPDM), promethazine hydrochloride (PH), diethazine hydrochloride (DH), prochlorperazine maleate (PCPM) and chlorpromazine hydrochloride (CPH) as reversible indicators.

EXPERIMENTAL

Reagents and apparatus

Indicator solutions. Aqueous solutions (0.2% w/v) of TFP, BPDM, PH, DH, PCPM and CPH were prepared and stored in amber bottles. BPDM and PCPM were dissolved in hot water (60°C).

Approximately 0.1 N solutions of ascorbic acid (containing no EDTA or formic acid [4], hydroquinone [4], metol [5] and chloramine-T [6]) were prepared and standardized by the accepted methods. All solutions were

stored in amber bottles and working solutions were made by suitable dilution of the standardized stock solutions. All reagents used were of AnalaR grade.

A Beckman Model DB spectrophotometer with 1-cm silica cells was used.

Determination of molar absorptivity of TFP, BPDM, PH, DH, PCPM and CPH

An aliquot (3 ml) of the 0.2% indicator solution and 10 ml of 10 M phosphoric acid (for BPDM, PH or CPH) or 15 ml of 10 M phosphoric acid for TFP, DH or PCPM) were transferred to a 25-ml volumetric flask. After addition of 2 ml of cerium(IV) sulphate solution containing 100—250 μg of cerium(IV), the solution was diluted to the mark with doubly-distilled water. The absorbance was measured at 502, 514, 512, 516, 526 and 528 nm for TFP, BPDM, PH, DH, PCPM and CPH, respectively, against a similar reagent blank. The arithmetic mean of the absorbance readings of four different concentrations of the oxidized forms of the indicators was taken to calculate the molar absorptivity.

Titration of 0.1—0.005 N hydroquinone

Dilute the hydroquinone solution (20 ml) and 7 ml of 10% potassium bromide solution to 40 ml with enough sulphuric, hydrochloric or acetic acid to give a concentration of 0.2 M sulphuric, 0.3 M hydrochloric or 2.0 M acetic acid at the end-point. Titrate the mixture with 0.1—0.02 N chloramine-T solution to the appearance of an orange-red colour, using 1 ml of 0.2% TFP, BPDM, PH, DH, PCPM or CPH indicator near the end-point (after 95% titration). For the titration of 0.01—0.005 N hydroquinone (10 ml), use 3 ml of 10% potassium bromide, dilute to 25 ml and titrate with 0.01—0.005 N chloramine-T to the appearance of a pink colour, adding 0.5 ml of the indicator towards the end-point.

Titration of 0.1—0.005 N metol

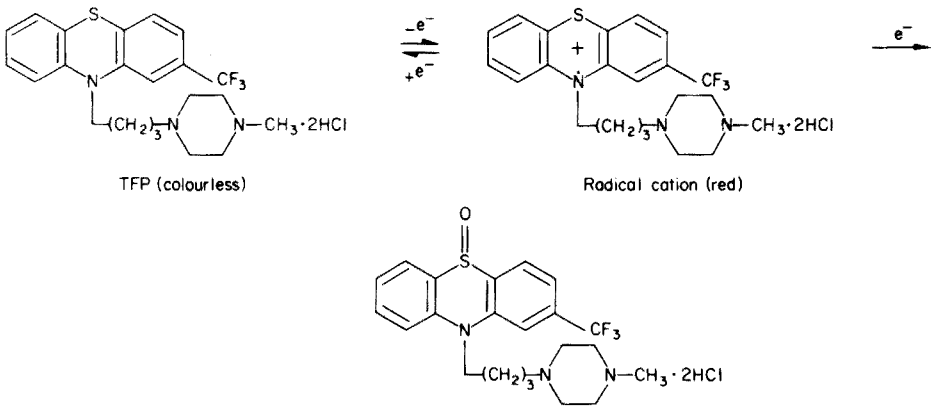
To an aliquot of 0.1—0.02 N metol solution, add 7 ml of 10% potassium bromide solution, and dilute to 40 ml with sulphuric, hydrochloric or acetic acid to give a concentration of 0.4 M sulphuric acid or 0.3 M hydrochloric or acetic acid at the end-point. Titrate the mixture with 0.01—0.02 N chloramine-T, using 1 ml of 0.2% TFP, BPDM, PH or DH indicator near the end-point (after 95% titration). For the titration of 0.01—0.005 N metol solution (10 ml), add 3 ml of 10% potassium bromide, dilute to 25 ml and titrate with 0.01—0.005 N chloramine-T, adding 0.5 ml of the indicator towards the end-point.

Titration of 0.1—0.05 N ascorbic acid

To the ascorbic acid solution (20 ml), add 7 ml of 10% potassium bromide, and dilute to 40 ml with enough sulphuric, hydrochloric or acetic acid to give a concentration of 0.2 M sulphuric acid, 0.4 M hydrochloric acid or 3.0 M acetic acid at the end-point. Titrate the mixture with 0.1—0.05 N chloramine-T solution as described for hydroquinone.

RESULTS AND DISCUSSION

TFP, PH, DH, PCPM and CPH are highly soluble in water giving colourless solutions; BPDM gives a light yellow aqueous solution. The aqueous solutions are stable for about 2 days at room temperature (27°C) and about 2 months at low temperature (6°C). The indicators undergo 1-electron reversible oxidation to a red intermediate which is believed to be a radical cation [7]. The radical cation is further oxidized irreversibly by excess of oxidant to a colourless sulphoxide [8]. The mechanism of oxidation of TFP can be represented by Scheme I. The mechanism of oxidation of BPDM, PH, DH, PCPM and CPH is similar to that of TFP.



TFP (colourless) Radical cation (red)

Sulphoxide (colourless)

The molar absorptivities of TFP, BPDM, PH, DH, PCPM and CPH are, respectively, 16.0, 20.7, 20.0, 20.9, 25.4 and 22.7 $\times 10^3$ l mol^{-1} cm^{-1}. These values indicate that the indicators should be very sensitive and should require only small indicator correction. Phosphoric acid was selected as the reaction medium to stabilize the coloured radical cation of TFP, BPDM, PH, DH, PCPM and CPH. Cerium(IV) sulphate was chosen as oxidant, because it is quantitatively reduced to cerium(III) sulphate giving an equivalent amount of coloured radical cation in the presence of a large excess of the indicator [9].

The redox potential of chloramine-T in 0.5 M sulphuric acid is 1.52 V [10], and decreases from 1.139 V in a solution of pH 0.65 to 0.614 V at pH 9.7 [11]. The redox potentials of all six indicators are reported elsewhere.

Titration of hydroquinone

Chloramine-T has not been used previously for the determination of hydroquinone. In acidic media containing potassium bromide, it produces bromine which oxidizes hydroquinone to quinone instantaneously. A simple but accurate titration of hydroquinone with TFP, BPDM, PH, DH, PCPM and CPH as redox indicators can be developed on this basis.

None of the six indicators gives colour change in acidic medium less than 0.1 M in hydrochloric or sulphuric acids or 0.3 M in acetic acid. All give a colour change at the equivalence point from light yellow to orange-red in the titration of 0.1—0.02 N hydroquinone and from very light yellow to pink in the titration of 0.01—0.005 N hydroquinone. The colour change from very light yellow to pink is sharper and brighter. TFP, BPDM, PH and DH give sharp colour changes in 0.2—2.0 M hydrochloric acid, whereas PCPM and CPH give good end-points only in the titration of 0.1—0.02 N hydroquinone in 0.2—0.5 M hydrochloric acid. At higher acidities, higher results are obtained in the titration of 0.1—0.05 N hydroquinone and low results in the titration of 0.1—0.005 N hydroquinone. The orange-red colour is stable for about 6 min and the pink colour for about 15 min. For the titration of 0.1—0.005 N hydroquinone, TFP and BPDM give sharp end-points in 0.1—1.5 M sulphuric acid; PH and DH in 0.1—1.0 M sulphuric acid; PCPM in 0.1—0.25 M sulphuric acid; and CPH in 0.1—0.4 M sulphuric acid solutions. Sluggish end-points are obtained at higher acidities. The orange-red colour is stable for about 5 min and the pink colour for about 10 min. TFP, BPDM, PH and DH give a sharp colour change at the end-point in 2—4 M acetic acid and CPH in 1—4 M acetic acid solutions. PCPM functions only in the titration of 0.01—0.005 N hydroquinone in 1.0—3.0 M acetic acid solution. Premature end-points are obtained at higher acidities. The orange-red colour is stable for about 4 min and the pink colours obtained with PH, DH, PCPM and CPH are stable for about 30 min, while those with TFP and BPDM are stable for only 5 min.

The indicator corrections are almost negligible in the titration of 0.1—0.05 N hydroquinone. The titration results presented in Table 1 compare favourably with those obtained potentiometrically with cerium(IV) sulphate.

Influence of bromide concentration. The minimum quantity of potassium bromide required in the titration of 0.1—0.02 N hydroquinone is 0.7 g in a total volume of 60 ml. In the titration of 0.01—0.005 N hydroquinone, 0.3 g of bromide is necessary in a total volume of 35 ml. Higher quantities of bromide (1—10 g) do not affect the titration values.

Influence of indicator concentration. At least 1.0 ml of 0.2% TFP, BPDM, PH, DH, PCPM or CPH is necessary for proper indicator action in the titration of 0.1—0.05 N hydroquinone. Higher concentrations (>2.0 ml) cause premature end-points. In the titration of 0.01—0.005 N hydroquinone, 0.5 ml of 0.2% indicator solution is required in a total volume of 35 ml. More than 1.5 ml gives high results.

For this titration, PH and DH are recommended as the most sensitive indicators.

Titration of metol

Metol (*N*-methyl-*p*-aminophenol sulphate) has not previously been determined with chloramine-T. The optimum conditions for the titration with TFP, BPDM, PH and DH as redox indicators are reported here. Metol is

TABLE 1

Determination of hydroquinone, metol and ascorbic acid with chloramine-T in the presence of BPDM, TFP, PH, DH, PCPM and CPH indicators

Reductant taken (mg)	Reductant found[a] (mg)	Relative error (%)	S.d.
Hydroquinone			
112.35	112.49	+0.13	0.0740
85.50	85.36	−0.16	0.0666
55.38	55.48	+0.18	0.0764
10.47	10.45	−0.19	0.0122
5.54	5.53	−0.18	0.0089
2.23	2.23	0.00	0.0070
Metol			
156.02	155.56	+0.27	0.0357
80.49	80.27	−0.27	0.0151
40.53	40.62	+0.22	0.0090
15.13	15.10	−0.20	0.0051
8.64	8.66	+0.23	0.0022
4.44	4.45	+0.23	0.0013
Ascorbic acid			
166.28	166.43	+0.09	0.0157
144.54	144.67	+0.09	0.0130
122.41	122.35	−0.05	0.0060
88.11	88.16	+0.06	0.0050
60.41	60.46	+0.08	0.0047
40.57	40.54	−0.07	0.0035

[a]Average of 5 determinations.

quantitatively oxidized to *N*-methyl-*p*-quinonimine by bromine liberated by chloramine-T from potassium bromide in hydrochloric, sulphuric or acetic acid medium; a 2-electron reaction is involved. The indicators do not function at acidities lower than 0.3 M sulphuric, 0.1 M hydrochloric or acetic acid. Stoichiometric results are obtained in 0.3—0.6 M sulphuric acid for TFP and BPDM, and in 0.3—0.5 M sulphuric acid for PH and DH. Under the conditions, all four indicators give sharp colour changes at the equivalence point, but high results are obtained at higher acidities. TFP and BPDM give sharp end-points in 0.1—0.7 M hydrochloric acid, and PH and DH in 0.1—0.5 M hydrochloric acid solutions. Premature end-points are obtained at higher acidities. End-points are good in 0.1—1.5 M acetic acid medium, but the results are high at higher acidities.

All four indicators give a sharp colour change from light yellow to orange-red (stable for 40—60 s) in the titration of 0.1—0.02 N metol and from very light yellow to pink (stable for 2—3 min) in the titration of 0.01—0.005 N metol. The effects of the concentrations of bromide and indicator are similar to those described for the titration of hydroquinone.

The indicator correction is almost negligible in the titration of 0.1—0.02 N metol. The titration results given in Table 1 compare favourably with potentiometric values obtained with cerium(IV) sulphate. The indicators recommended for this titration are TFP and PH in sulphuric acid; BPDM and PH in hydrochloric acid, and BPDM in acetic acid medium.

Titration of ascorbic acid

Variamine blue [2] and cacotheline [3] have been proposed as reversible indicators for the titration of ascorbic acid with chloramine-T. Variamine blue works only in 0.4 M acetic acid medium, giving sluggish end-points at other acidities. Cacotheline functions in sodium acetate—hydrochloric acid buffer of pH 4.5 but the end-points are poor.

TFP, BPDM, PH, DH, PCPM and CPH indicators give sharp reversible colour changes from colourless to pink (orange-red by TFP) at the equivalence point. They do not function in sulphuric, hydrochloric or acetic acid media less than 0.1 M. PH and DH give sharp end-points in 0.1—1.5 M sulphuric acid, BPDM and TFP in 0.1—2.0 M sulphuric acid, PCPM in 0.1—0.2 M sulphuric acid and CPH in 0.1—1.0 M sulphuric acid solutions. The stabilities of the end-point colour increase with increasing acidity: 40—55 min for CPH in 0.1—1.0 M sulphuric acid, 15—70 min and 35—100 min for PH and DH in 0.1—1.5 M sulphuric acid, and 10—30 min and 10—100 min for BPDM and TFP in 0.1—2.0 M sulphuric acid. PH, DH, BPDM and TFP give good end-points in 0.2—3.0 M hydrochloric acid, PCPM in 0.2—3.0 M hydrochloric acid and CPH in 0.2—2.0 M hydrochloric acid solutions. The end-point colours of PH, BPDM and TFP are stable for about 30 min, those of DH and CPH for about 60 min. The pink colour of PCPM is stable for about 15 s in hydrochloric or sulphuric acid medium. At higher acidities, late end-points are obtained.

PH, DH, TFP and CPH give very sharp and precise end-points in 3.0—4.5 M acetic acid, BPDM in 1.5—3.0 M acetic acid, and PCPM in 2—4 M acetic acid solutions. At lower and higher acidities, high results are obtained. The end-points obtained with PH, DH, CPH, PCPM, BPDM and TFP are stable for 15, 15, 1.2, 0.6, 0.1 and 0.1 h, respectively.

The indicator corrections are almost negligible, and the effects of concentration of indicators and bromide are similar to those described for titrations of hydroquinone. PH and DH are the most sensitive indicators in this titration.

Interferences. Ten-fold molar amounts of oxalic, citric, tartaric, succinic, acetic and malic acids, glucose, fructose, sucrose, starch and acetone do not interfere in the determination of ascorbic acid. The results presented in Table compare favourably with those obtained by the iodimetric method [12].

Comparison with other indicators. All six indicators are superior to variamine blue and cacotheline. They give sharper and brighter end-points and function over a wider range of different acidities and the reversal of their colour changes at the equivalence point can be repeated more often than with the earlier indicators.

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Short Communication

DETERMINATION OF MOLYBDENUM IN IRON AND STEELS BY U.H.F. PLASMA TORCH SPECTROMETRY

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Many spectrophotometric and atomic absorption spectrometric methods have been utilized to determine traces of molybdenum in iron and steels [1—5]. These procedures generally involve solvent extraction or the use of a nitrous oxide—acetylene flame, but their sensitivity is not very high. It has been shown that u.h.f. plasma torch spectrometry is very suitable for the determination of chromium [6] and manganese [7] in iron and steels. This communication describes a detailed investigation of the determination of molybdenum by this technique. A rapid, simple method for the determination of molybdenum in iron and steels is outlined.

Experimental

Standard molybdenum solution. Molybdenum metal (99.9%) was dissolved in aqua regia; the solution was evaporated almost to dryness, the residue dissolved in 6 M hydrochloric acid, and the solution diluted with 6 M hydrochloric acid to contain 10 mg Mo ml^{-1}. This solution was diluted as required just before use.

All acids and salts and other metals were of reagent grade.

Apparatus. A 2450-MHz Hitachi u.h.f. plasma torch spectrometer 300 was used, as described previously [6]. Argon was used as the plasma-forming gas and sheath gas.

Working conditions. Factors such as the field and anode current, and flow rates of the plasma-forming and sheath gases, which fundamentally affect the line intensity of molybdenum in the u.h.f. plasma torch, were examined in the same way as for chromium or manganese [6, 7]. The relationships between the line intensity and these factors except the flow-rate of plasma-forming gas were almost the same as for chromium and manganese. As mentioned previously, the effect on the line intensity of the flow-rate of plasma-forming gas, which carries the sample, depended on the composition of the sample solution. The Mo line intensity showed a maximum at 3.5 l min^{-1} for the plasma-forming gas when a solution containing 7 ppm of molybdenum in distilled water was used (Fig. 1). But when the solution contained 10 mg Fe ml^{-1} in 0.5 M hydrochloric acid, it showed a maximum at 3.0 l min^{-1}.

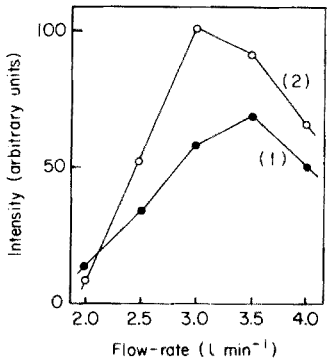


Fig. 1. Relation between line intensity and the flow-rate of plasma-forming gas. 7.0 μg Mo ml^{-1} in distilled water (1) and in 0.5 M HCl containing 10 mg Fe ml^{-1} (2).

The optimal working conditions for molybdenum were as follows: frequency, 2450 MHz; flow-rate of plasma-forming gas, 3.0 l min^{-1}; flow-rate of plasma-sheath gas, 3.5 l min^{-1}; field current, 370 mA; anode current, 270 mA; entrance slit width, 30 μm; exit slit width, 50 μm.

Results and discussion

Several spectral lines of molybdenum may be used. The Mo I 379.825-nm line was most intense (3.26 eV) [8], but there was apparent spectral interference from the Fe I 379.851-nm line. The Mo I 390.296-nm line (3.17 eV) was also interfered with by the Fe I 390.295-nm line. Therefore, the Mo I 386.411-nm line (3.20 eV) was adopted. The relative plasma-torch intensities for the 379.825, 386.411 and 390.296-nm lines are 150, 122 and 92, respectively, in arbitrary units.

The spatial distribution of the chromium and manganese emissions in the plasma torch depend on the composition of the sample solution [6, 7]. Therefore the spatial distribution of the Mo I 386.411-nm line was investigated in the presence of iron in 3 M hydrochloric acid and in the absence of iron. The intensity was greatest at the positions 1.5 mm horizontally from the centre of the plasma. Though the intensity distribution of chromium shifted in the presence of a large amount of iron [6], that of molybdenum, did not shift, similarly to manganese [7].

Effects of acids and interfering elements. The effects of common mineral acids in the range 1—5 M were investigated. The emission intensity decreased significantly with increasing concentration of acid, but remained constant for 2.0—5.0 M hydrochloric acid; at such acidities, the emission intensity for 5.0 μg Mo ml^{-1} solutions was depressed by about 25% compared to pure aqueous solutions. Nitric and sulfuric acids had large depressive effects and were considered unsuitable for use.

The effects on molybdenum emission intensity of the elements usually found in iron and steels were examined; solutions containing 5.0 μg Mo ml^{-1}

and 200 $\mu g\ ml^{-1}$ of the other ions were used, because this concentration ratio (1:40) is usually the greatest encountered in steel analysis. The presence of Co, Ti, Mn, Ni or Cu increased the intensity but Al, Sn, Nb, Zn and W decreased it (Table 1).

The effects of large amounts of iron are shown in Fig. 2. The intensity at the molybdenum line swiftly increased with an increase in iron concentration up to 5 mg Fe ml^{-1} and then became almost constant in the concentration range 5—15 mg Fe ml^{-1}, in 0.5 M hydrochloric acid when the flow-rate of plasma-forming gas was 2.5 or 3.0 l min^{-1}. In contrast, the intensity decreased with an increase in iron concentration above 5 mg ml^{-1}, when the flow-rate was 3.5 l min^{-1}.

At these flow-rates of plasma-forming gas, the effects of hydrochloric acid concentration were examined in the presence and absence of iron. In the absence of iron, as mentioned above, the intensity remained approximately constant for the range 2.0—5.0 M hydrochloric acid. But when the sample solution contained a large amount of iron, the intensity decreased with increasing concentration of acid (Fig. 3). To determine molybdenum in iron and steels, Fig. 3 indicates that a flow-rate of 3.0 l min^{-1} should be suitable, at a fixed concentration of hydrochloric acid.

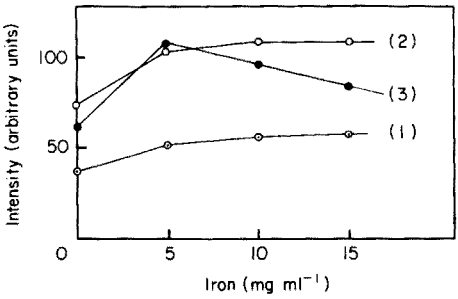
It was reported earlier for chromium and manganese that a large amount of iron prevented interferences from small amounts of other elements [6, 7]. In this study a large amount of iron was also found to prevent the interference

TABLE 1

Effect of other elements on molybdenum emission intensity at 386.411 nm (5.0 μg Mo ml^{-1})

Element	In 0.5 M HCl		In 0.5 MHCl + 10 mg Fe ml^{-1}	
	Mo found ($\mu g\ ml^{-1}$)	Error (%)	Mo found ($\mu g\ ml^{-1}$)	Error (%)
As	5.0	0	5.0	0
Cr	5.1	2	5.0	0
Co	5.3	6	5.0	0
Ti	5.9	18	4.6[c]	−8
Mn	6.1	22	5.0	0
Ni	6.1	22	5.0	0
Cu	6.3	26	5.0	0
V	4.9	−2	5.0	0
Al	4.6	−8	4.9	−2
Sn	4.3	−14	5.0	0
Nb	4.0	−20	4.4[b]	−12
Zn	3.9	−22	5.0	0
W	3.5	−30	5.0	0

[a] 200 $\mu g\ ml^{-1}$. [b] 50 μg Nb ml^{-1} gave 4.9 μg Mo ml^{-1}.
[c] 50 μg Ti ml^{-1} gave 4.9 μg Mo ml^{-1}.



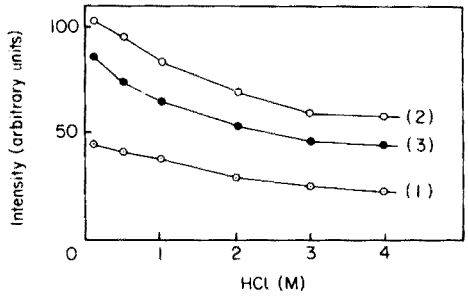


Fig. 2. Effect of iron on molybdenum intensity at 386.411 nm for 7.0 μg Mo ml^{-1} in 0.5 M HCl; flow-rate of plasma-forming gas: (1) 2.5 l min^{-1}; (2) 3.0 l min^{-1}; (3) 3.5 l min^{-1}.

Fig. 3. Effect of hydrochloric acid on molybdenum intensity at 386.411 nm in the presence of iron. 7.0 μg Mo ml^{-1}, 5 mg Fe ml^{-1}; flow-rate of plasma-forming gas: (1) 2.5 l min^{-1}; (2) 3.0 l min^{-1}; (3) 3.5 l min^{-1}.

of elements such as Ti, Mn and Ni. Table 1 shows that, for the determination of 5.0 μg Mo ml^{-1} in the presence of 10 mg Fe ml^{-1} in 0.5 M hydrochloric acid, 200 μg ml^{-1} of As, Cr, Co, Ni, Mn, Sn, Zn, V, W and Al, and 50 μg ml^{-1} of Nb and Ti did not interfere.

Murayama [9], describing u.h.f. plasma-torch experiments with rare-earth elements, mentioned that the emission intensities of some rare earths such as samarium, lanthanum and neodymium were enhanced by the presence of alkali metals and the detection limits for these elements were improved. Accordingly, the effect of addition of alkali metals on the molybdenum intensity was examined. Sodium and potassium enhanced the intensity, but the sensitivity was improved only 1.3 times in the presence of 50-ppm alkali metal (Fig. 4). The intensity distribution across the plasma torch was not affected by the alkali metals.

A large amount of iron is useful as a radiation buffer for the elimination of interferences from small amounts of other elements and for the enhancement of the emission intensities of the elements (Mo, Cr, Mn) being determine

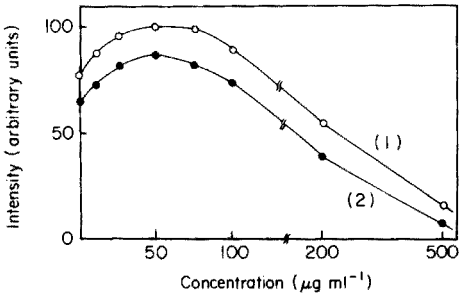


Fig. 4. Effect of alkali metal salts on molybdenum intensity at 386.411 nm. 5 μg Mo ml^{-1}; (1) sodium; (2) potassium (as chlorides).

Thus, it may be considered that the addition of a large amount of another element converts the plasma torch to thermodynamic equilibrium, because the electronic energy in the plasma is converted to thermal energy by collisions between the electrons and the atoms and molecules introduced into the plasma by the addition of a large amount of another element. So the electron temperature decreases and the gas temperature may increase, when a large amount of iron is added [7].

Analytical procedure and results. Calibration curves for molybdenum, prepared in the presence of iron (5—15 mg ml^{-1}) in 0.5 M hydrochloric acid under the recommended instrumental conditions were linear up to 50 μg Mo ml^{-1}. The coefficient of variation was 1.1% (11 results) for the mid-point of the calibration curve (25 μg Mo ml^{-1}).

Based on these results, the following procedure for the determination of molybdenum in iron and steels was established.

An accurately weighed sample (0.5—1.5 g) was dissolved in 15 ml of aqua regia by heating. The solution was evaporated almost to dryness, the residue was dissolved in 20 ml of 2.5 M hydrochloric acid, and the solution was transferred to a 100-ml volumetric flask and diluted to volume with distilled water. (When large amounts of silicon were present, they were removed by treatment with hydrofluoric acid in a platinum crucible. After filtering the sample solution, the filter paper was washed with 2.5 M hydrochloric acid and the washings added to the main solution. In this case, the total concentration of hydrochloric acid had to be kept constant.)

The results obtained for the determination of molybdenum in iron and steels (Table 2) are in satisfactory agreement with certificate values.

TABLE 2

Analytical results for standard iron and steels

Sample	Mo found (%)	Certificate value (%)	C.v. (%)
J.S.I.S.[a] 159-2	0.014_0	0.014	—
J.S.I.S. 160-2	0.15_6	0.16	—
J.S.I.S. 161-2	$0.31\frac{b}{3}$	0.31	1.2

[a]Japanese Standard of Iron and Steels. [b]Average of 7 determinations (range 0.307—0.318).

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Short Communication

A SIMPLE OXIDATION PROCEDURE FOR BIOLOGICAL MATERIAL PRIOR TO ANALYSIS FOR MERCURY

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The determination of mercury in biological material has always proved problematical because of the difficulty of completely oxidizing the matrix while quantitatively retaining the mercury. Specific oxidation techniques developed to destroy organic material prior to mercury analysis [1—4] are incapable of oxidizing some materials (e.g. shellfish, eggs) completely. The reason for this is the low temperature necessary during the oxidation stage to avoid mercury volatilization. This report describes a modification of a technique used for the determination of mercury in coal [5]. The sample is mixed with an inert matrix and burned in a stream of oxygen inside a combustion tube. The exhaust gases are passed through acidified potassium permanganate to remove entrained mercury. The mercury in solution is analysed by the cold vapour technique of Hatch and Ott [6], after preconcentration by amalgamation with silver [7].

Experimental

Reagents. To provide a mercury-free matrix, industrial sand was heated in a muffle furnace at 800°C for 48 h to remove mercury, ground to a $<84\ \mu m$ powder, reheated at 800°C for 24 h, cooled, broken up, and stored in a dark glass jar.

A 5% (w/v) solution of low-mercury potassium permanganate, a 14% (v/v) solution of sulphuric acid, and a 20% (w/v) solution of hydroxylammonium hydrochloride were prepared in distilled water.

Mercury absorbing solution. Tests were made for the suitability of different permanganate solutions. Known amounts of mercury-saturated air were injected into the oxygen stream flowing through a 160-mm absorption cell aligned in the optical path of an atomic absorption spectrometer, and a reference curve was constructed (Fig. 1A). Then, the oxygen stream was passed through a gas wash bottle containing distilled water (Fig. 1B) or various acidified potassium permanganate solutions (Fig. 1C—E), and the calibration was repeated. The mixture of 25 ml of 5% potassium permanganate and 50 ml of 14% sulphuric acid (Fig. 1E) absorbed at least 2000 ng Hg;

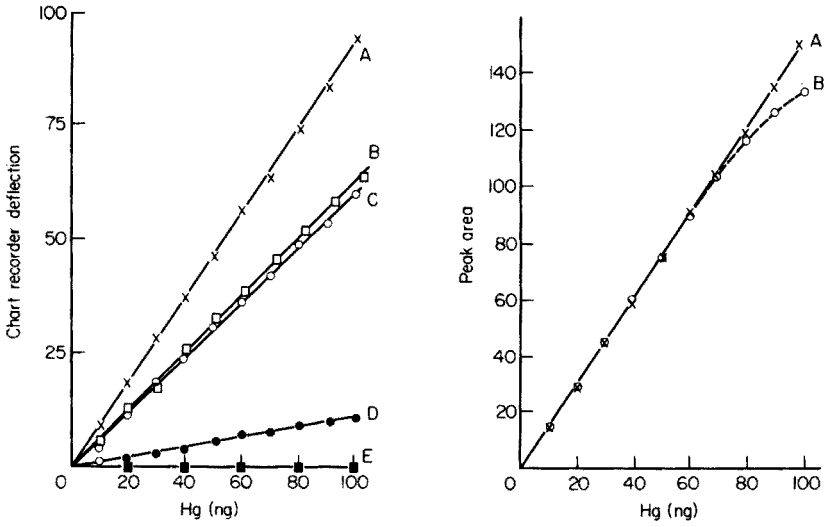

Fig. 1. A. Reference calibration. B. 75 ml of distilled water. C. 25 ml of 0.15% potassium permanganate solution plus 50 ml of 1.5% sulphuric acid. D. 25 ml of 1.5% potassium permanganate solution plus 50 ml of 15% sulphuric acid. E. 25 ml of 5% potassium permanganate solution plus 50 ml of 14% sulphuric acid.

Fig. 2. Effectiveness of silver wool as a mercury absorber. A. Direct analysis. B. Analysis after amalgamation onto silver wool.

once absorbed, the mercury was stable for 48 h. The release of mercury from this solution, after reduction with hydroxylammonium hydrochloride, was equivalent to that from distilled water. This indicated that there was no interference from manganese or potassium. This solution was used throughout further experiments.

Amalgamation trap. Concentrations of mercury in the samples were extremely low, necessitating a preconcentration step [7]. Silver wool (500 mg) was packed into a quartz tube and heated at 700°C for 12 h to remove any mercury. The cooled tube was placed inside a resistance furnace and a known quantity of mercury-saturated air was passed through it, to form the silver amalgam. The tube was then heated at 700°C for 10 s and the released mercury vapour passed into the absorption cell of the spectrometer. The amalgam breaks at 500°C; 700°C releases the mercury but does not melt the silver. Absorption peaks were recorded, and the peak areas were calculated and compared with similar peaks obtained by direct injection of mercury vapour (Fig. 2). Peak areas and not peak heights were compared because of differences between peak shapes from the two experiments. Amounts of mercury of less than 60 ng are completely amalgamated by the silver wool. A smaller sample should be used if this quantity is exceeded.

Analysis of artificial standards. Samples (500 mg) of freeze-dried, homogenized shellfish tissue were spiked with known amounts of either methyl-

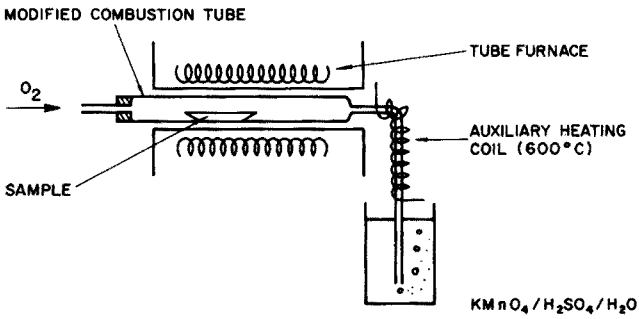

Fig. 3. Diagram of thermal oxidation apparatus.

mercury chloride or ethylmercury chloride and mixed with 2 g of mercury-free matrix. A moderator such as sand was necessary to avoid spontaneous combustion of the sample and hence an explosion inside the tube. Each sample was transferred to a 100-mm long ceramic boat and placed inside a modified silica combustion tube in the centre of a Gallenkamp FTH-350-010C tube furnace. The furnace was preheated to 700°C (Fig. 3). Oxygen was passed over the sample and the exhaust gases were bubbled through a solution of 25 ml of 5% potassium permanganate and 50 ml of 14% sulphuric acid until combustion was complete; an oxygen flow rate of 750 ml min^{-1} gave complete combustion of the sample without production of smoke.

The acidified potassium permanganate solution (10 ml) was transferred to a Dreschel bottle; 100 ml of distilled water was added. Hydroxylammonium chloride solution (2 ml) was added to reduce the permanganate; then 2 ml of 20% tin(II) chloride in 20% (v/v) hydrochloric acid was added to reduce the inorganic mercury to elemental mercury, which was purged from the solution with argon as carrier gas to form an amalgam with silver wool. The amalgam was subsequently dissociated thermally, and the released mercury was passed through the absorption cell aligned in the optical path of the

TABLE 1

Recovery of added mercury

Sample	Hg compound added	Hg added (ng)	Hg in sample[a] (ng)	Recovery (%)	s_r (%) (n = 20)
Matrix	—	—	0.0	—	—
Matrix + 0.5 g shellfish	—	—	39.0	—	5.0
Matrix + 0.5 g shellfish	Methylmercury chloride	10	47.9	89	7.2
Matrix + 0.5 g shellfish	Ethylmercury chloride	10	48.3	93	4.6

[a]All samples corrected for a reagent blank of 3.0 ng Hg; s_r 7.1%.

TABLE 2

Comparison of IAEA values with those obtained by this method

Sample	IAEA value ($\mu g\ kg^{-1}$)	s^a	n	This method ($\mu g\ kg^{-1}$)	s	n
SP-M-1 (Sea grass)	337	111	23	262	10	10
MA-A-1 (Copepod)	255	71	17	202	8	10
A6 (Fish soluble)	78	24	14	102	4	10

[a]Standard deviation for n determinations.

spectrometer. Absorbance readings were compared with those from artificial inorganic standards and converted to mercury concentrations in the original samples (Table 1). Even with relatively volatile organomercury compounds at extremely low levels, the recovery of added mercury was good and reproducible.

Analysis of IAEA biological tissues. The International Atomic Energy Agency distributes samples of biological materials to laboratories for intercalibration exercises to standardize the materials. Three IAEA samples, SP-M-1 (sea grass), MA-A-1 (copepod), and A6 (fish solubles) were analysed for mercury by the method described. The concentrations obtained are compared with those reported by the IAEA in Table 2.

The values reported by the IAEA, even after rejecting outliers, have a relative standard deviation of ca. 30% and, as such, have not been given "probable concentration" status. Nevertheless, it may reasonably be assumed that the mercury concentrations for these samples lie within the limits given in Table 2. All results obtained in the present study lie within these concentration ranges and the relative standard deviation for 10 determinations is ca. 5% in each case.

This method is fast, reproducible and inexpensive; it can be applied to the determination of mercury in both wet and dry tissues without sample pretreatment.

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Short Communication

THE SPECTROPHOTOMETRIC DETERMINATION OF TRACES OF CHLORIDE IN CORROSION PRODUCTS

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There are numerous spectrophotometric methods of determining micro-amounts of chloride in boiler water containing small amounts of other anions and cations that do not interfere. A solution obtained by dissolving the corrosion products of various metals in aqueous solutions may, however, hold trace amounts of chloride and 100-fold greater amounts of cations, some of which may invalidate the values given by a particular method. Of all the methods in the literature, the most sensitive and least subject to interferences from iron corrosion products is based on the exchange reaction between chloride and $Hg(SCN)_2$, with subsequent spectrophotometric determination of SCN^- as iron thiocyanate [1—4]. This method, however, would not be suitable for the analysis of copper corrosion products, which can form thiocyanates. The interference of copper may be eliminated by adopting Davies' method [5]; dissolution of the corrosion products in chromic acid is followed by distillation of the hydrochloric acid and its final measurement by the thiocyanate method. It is not possible to determine the other corrosion products. Furthermore, instability of the iron thiocyanate solutions does not allow precise measurements [6]. Consideration has not been given to methods based upon the exchange reaction between phenylmercury nitrate and chloride in solution; this method is too laborious [7, 8] and does not permit great accuracy. The mercury cyanide [9] method is also inadequate because of interferences by various cations, (e.g. Cu^{2+}) although measurement in the ppb range is possible.

The metathesis reaction between $AgIO_3$ [10—12] or $HgIO_3$ [13—15] and chloride with subsequent iodimetric measurements of released IO_3^- was thought to be more precise, though slightly less sensitive. Although interferences are not numerous, and some can be easily eliminated (e.g. reducing substances through oxidation with HNO_3), others (e.g. copper) may cause considerable difficulties. Despite this drawback, it was decided to endeavour to increase the sensitivity of this method and eliminate the copper interferences. Of the two reagents recommended (i.e. $AgIO_3$ and $HgIO_3$), $AgIO_3$ was preferred because AgCl resulting from the reaction between $AgIO_3$ and

Cl^- ions in the solution will have ten times lower solubility [15] than the $HgCl_2$ from reaction with $HgIO_3$.

Experimental

Equipment. Absorbance measurements were made with a IASCO Uvidec-5 spectrophotometer.

Silver iodate (B.D.H.). Dry in air at 100°C to constant weight, grind in an agate mortar, screen to pass a 200-mesh sieve, and keep in a desiccator.

Zerolit 225 (B.D.H.). Pack in a column (25 mm i.d., 600 mm high); condition the resin with HNO_3 (1 + 1) and repeatedly flush with deionized water.

Procedure. Acidify the solution (10—100 ml) containing 1—50 μg Cl with 0.1 ml of 65% HNO_3 and heat to 80—90°C for 10 min. After cooling, adjust with 0.1 M NaOH to pH 4.7 and pass the solution through the ion-exchange column. Collect the cation-free liquid and concentrate to 1 ml on a water bath at 50—60°C (care must be taken to prevent the solution going to dryness). Mix the concentrated solution with 19 ml of methanol and 60 mg of $AgIO_3$ by stirring for 3 min. The same time and manner of stirring must be used in all cases. Filter the opalescent solution (Schleicher blue band paper), and collect in a volumetric flask containing 200 ml of the aqueous solution prepared for iodimetric determination of the free iodate.

The solution for spectrophotometric analysis contains 5 ml of aqueous 0.5% (w/v) starch (prepared daily), 1 ml of glacial acetic acid and 2 g of KI. The latter reagent is added immediately before colorimetric development. The solution must be kept at 18°C. Measure the absorbance (2-cm cell) at 590 nm, 30 min after color formation; the intensity is stable for at least 2 h.

Results and discussion

Two approaches were adopted in the development of the method: (1) a study of the most suitable means of increasing the sensitivity to determine chloride in the ppb rather than the ppm range; (2) elimination of interferences. The poor sensitivity of the silver iodate method is essentially due to the high value of the "blank" resulting from the appreciable solubility of silver iodate in water; the quantity of iodate deriving from the solubility of $AgIO_3$ is equivalent to several ppm of chloride. The solubility of $AgIO_3$ was reduced by finding the optimum volume of methanol required to obtain an acceptable blank value. The optimum volumes are 1 ml of water to 19 ml of methanol. The metathesis reaction in this methanolic medium takes place according to the same kinetics and with the same yield as in water; the difference between the absorbances obtained in the presence and absence of chloride was constant for all water percentage values investigated (Fig. 1).

A study was also made of the optimum conditions for color development. The final dilution of the colored solution affects the absorption of the chromophore groups and, hence, the reproducibility of measurement (Table 1). It was therefore decided to operate with a final volume of 250 ml.

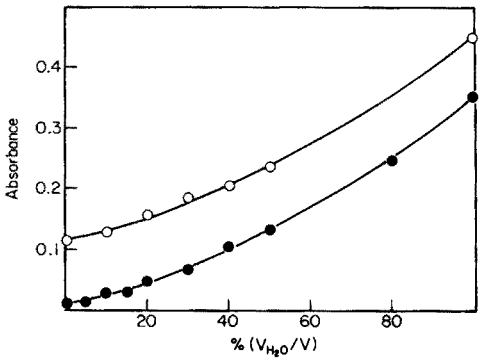

Fig. 1. $AgIO_3$ solubility versus % water in methanolic solutions. •H_2O + CH_3OH. ○H_2O + CH_3OH + 5 μg Cl^-.

Temperature also affects the precision [17]. Tests carried out at 18 and 25°C showed that the absorption intensity decreased slightly with increasing temperature; analysis is not recommended at temperatures higher than 18°C. The absorption intensity increases during the first 30 min, but further variation does not occur for at least 2 h.

Table 2 shows the recoveries of chloride obtained by following the above procedure without passing the solution through an ion-exchange resin. To remove the interferences caused by some cations, e.g. Cu^{2+}, Fe^{2+}, etc., consideration was given to precipitation (e.g. as hydroxides) and adsorption on an ion-exchange resin. Double precipitation of the cations as hydroxides does not allow complete recovery of the chlorides, which remain in the precipitate. Adsorption on Zerolit 225 resin led to satisfactory results (Table 3). The main difficulty encountered during development of the method was the choice of the optimum pH value for deionization. It was found that at least 95% of the cations are adsorbed on passage through the resin column at pH 4.7.

Conclusions. It is possible to determine ppb quantities of chloride, in the presence of a 50,000-fold quantity of copper or iron; over the whole concentration range, the error is ca. 5% and the precision better than ± 10%. For 4—20 μg Cl l^{-1}, the plot of absorbance vs. concentration was linear with slope $A = (0.0176 \pm 4.75 \times 10^{-7})\,c + (0.0181 \pm 2.26 \times 10^{-3})$ at 18°C: for

TABLE 1

Absorbance of solutions containing 25 μg Cl^-, 1 g KI and 5 ml of 0.5% starch in different final volumes: mean values of 8 analyses reported. λ = 590 nm; l = 2 cm.

Final volume (ml)	500	250	100
Absorbance	0.182	0.428	0.750
s	0.006	0.008	0.091

TABLE 2

Recovery data for chloride; mean values of 5 analyses reported

Cl^- present (ppm)	0.01	0.10	0.30	0.50	1.00
Cl^- found (ppm)	0.008	0.102	0.305	0.487	1.060
Error (%)	−20	+2	+1.7	−2.6	+6.0

TABLE 3

Recovery data for chloride; from a solution containing 5000 μg Cu^{2+} and 5000 μg Fe^{3+} in 100 ml; mean values of 5 analyses reported

Cl^- present (ppm)	Cl^- found			
	Precipitation as hydroxide	Error (%)	Resin adsorption pH 4.7	Error (%)
0.10	0.00	−100	0.009 ± 0.001	−10
0.50	0.30 ± 0.15	−40	0.475 ± 0.06	−5
1.00	0.73 ± 0.22	−27	0.995 ± 0.008	−0.5

20 − 100 μg Cl l^{-1} a linear plot defined by $A = (0.01625 \pm 8.05 \times 10^{-8})\, c + (0.0149 \pm 5.35 \times 10^{-3})$ was obtained at 18°C.

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Short Communication

SPECTROPHOTOMETRIC DETERMINATION OF TRACE AMOUNTS OF YTTRIUM IN SILICATES AFTER CATION EXCHANGE SEPARATION WITH DL-2-HYDROXYBUTYRIC ACID

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The geochemistry of trace elements can yield information which cannot be deduced unambiguously from geology, and has been applied for characterization of different magmas [1—6]. Yttrium contents, together with zirconium, niobium, titanium, phosphorus and chromium, differ in magma types and are independent of weathering and metamorphism, so that data on these elements is valuable. The determination of trace amounts of yttrium is one of the most important steps in characterizing magmatic rocks. Unfortunately yttrium is very difficult to determine. In the published data for rock standards, the values were obtained by x-ray fluorescence or optical emission spectrography, with a few data from mass spectrometry [7—10]. Neutron activation analysis is not useful for trace amounts of yttrium, probably owing to the short life of its isotopes; recently, encouraging results have been obtained with 10-MeV proton activation and direct γ-spectrometry [11], but such methods are not entirely satisfactory and are not readily available. Colorimetric methods are usually preferred in rock analysis laboratories; yttrium has a low sensitivity in a.a.s.

The colorimetric technique commonly employed [12—14] to determine total yttrium plus rare earths is based on the complexes formed with arsenazo-III (2,7-bis(2-arsenophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid). This reagent is not selective: coloured compounds are formed with the rare earths and zirconium, hafnium, iron, aluminium, and calcium [12—14]. When a determination of yttrium only is desired, the determination suffers from interferences from the silicate matrix (Ca, Fe and Al) and other trace components present. To avoid these errors, Voldet and Haerdi [14] proposed a standard addition method, and Kirillov et al. [13] employed solvent extraction.

These methods take into account only the interferences present when a total Y + rare earth elements group determination is required. Ion-exchange separation procedures have been employed by several authors [15—18] to separate yttrium and single rare earth elements from a synthetic mixture. Zeligman [15] and Nervik [16] adopted an ion-exchange pH-gradient elution method. Szlaurova et al. [17] presented a quantitative group separation for 15 radionuclides, including yttrium, by means of an anion-exchange resin with mixed solvent systems. Separation of the groups into individual components was achieved by changing the eluant and resin types. Seven ion-exchange columns were employed. Fenyo et al. [18] described a separation of lanthanides on ion-exchange paper SA-2 in α-hydroxyisobutyrate medium. Nevertheless, such methods ignored the presence of iron, calcium, aluminium and other trace elements present in the silicate matrix of rock samples and were thus subject to interference.

To eliminate the major elements (Fe, Al and Ca) in the silicate matrix an ion-exchange procedure based on the data presented previously [19, 20] has been used. Zirconium, hafnium and thorium can be completely eluted from the ion-exchange column with oxalic acid [21, 22]. The remaining ions (Y + rare earths) are eluted successively with 6 M HCl [20]. The sample thus obtained, containing yttrium and other interfering ions, is sorbed on a column filled with Dowex 50-X4 resin (ammonium form) and successively eluted with 0.24 M and 0.36 M DL-2-hydroxybutyric acid (HBA). The first fraction, from 0.24 M HBA buffered at pH 5, contains Lu, Yb, Tm, Er, and Ho and is discarded. The first fraction from 0.36 M HBA buffered at pH 5 (15 ml) is used for the determination of yttrium.

Experimental

Apparatus. The Beckman DK-2A recording spectrophotometer was equipped with 100-mm cells. Eppendorf micropipettes were used for micro-sampling.

Solutions. The HBA solutions (0.24 M and 0.36 M) are buffered at pH 5 with ammonium hydroxide (6 M). For the triethanolamine buffer, mix 15 ml of triethanolamine and 3.3 ml of 68% HNO_3, and dilute to 200 ml with deionized water. For the yttrium standard solution, weigh 50 mg of yttrium metal in a 250-ml beaker, add 0.6 M HCl until solution is complete, transfer to a 250-ml graduated flask and dilute to volume.

Ion-exchange column (a). Fill a borosilicate glass tube (22 mm i.d., with fused-in glass sinter of No. 3 porosity and a buret tap at the bottom) with Dowex 50W-X8 resin (200—400 mesh, H^+ form) to give a column height of 180 mm and wash it with 300 ml of 3 M HCl. Wash with deionized water until free from acid. Adjust the flow-rate to 3.0 ± 0.5 ml min^{-1} (giving a back-pressure of 360 mm Hg). Pretreat the column with 30 ml of 6 M HCl — 0.15% H_2O_2 solution.

Ion-exchange column (b). Fill a borosilicate glass tube (6 mm i.d., with fused-in glass sinter of No. 3 porosity, without buret tap) with Dowex 50W-X4 (200—

400 mesh, H^+ form) to give a column height of 110 mm. Convert the resin to the ammonium form with 1 M NH_4Cl, wash with deionized water, and pretreat with one column volume of 0.24 M HBA at pH 5.

Procedure. Weigh out 0.3000 ± 0.0001 g of finely powdered (120-mesh) sample in a nickel crucible. Add ca. 1.5 g of sodium peroxide, mix with the sample, and cover with another 1.5 g of sodium peroxide. Fuse over a bunsen burner on an asbestos board to obtain a black melt. Cool, and place the crucible, previously cleaned, in a beaker containing hot 6 M HCl—0.15% H_2O_2 solution, warming to obtain a clear solution; dilute to volume in a 250-ml volumetric flask.

Pass the sample solution through the first column. Elute the major elements of the silicate matrix with 250 ml of 3 M HCl in 20% ethanol. Wash the column with water until free from acid. With 150 ml of 0.5 M oxalic acid solution, elute Zr, Hf, and Th. Collect yttrium and rare earth elements by passing 300 ml of 6 M HCl through the column. Concentrate in a Teflon beaker and slowly evaporate to near dryness. Cool and add 10 ml of 0.24 M HBA solution. Stand overnight on a magnetic stirrer. Pass the solution obtained through the second exchange column. Pass 60 ml of the 0.24 M HBA solution and discard the eluate (this solution contains Lu, Yb, Tm, Er and Ho). Pass 15 ml of the 0.36 M HBA solution, and collect in a 25-ml graduated flask. Yttrium is contained in this fraction. Add 3 ml of arsenazo-III solution (0.15%) followed by 5 ml of triethanolamine buffer. Dilute to volume and stand for 1 h. Read at 570 nm in 10-cm cells.

Results and discussion

Fusion with sodium peroxide followed by hydrogen peroxide—hydrochloric acid leaching is a powerful method of dissolution for rocks and minerals [23]. At the trace level in silicate rocks, yttrium is present principally as a substitute for other major or minor elements.

After collection of the Y + rare earths group, yttrium was separated by developing, for column use, the technique studied by Fenyo et al. [18] for ion-exchange paper separation. The separation was developed colorimetrically (arsenazo-III) on solutions of single rare earths which were each passed through the column. The order of elution is shown in Fig. 1a. Because of the

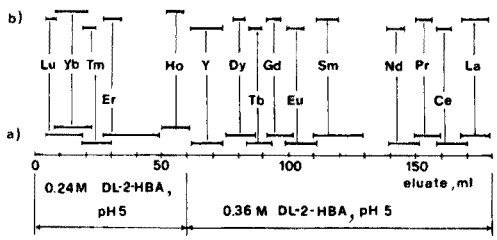

Fig. 1. Rare earths and yttrium elution schemes with DL-2-hydroxybutyric acid width at half peak height. (a) 1:1 element ratios (b) element ratios common in silicates.

relatively insensitive method used, the elution curve cannot be accurate and overlapping of the peaks may reasonably be expected. This is due, largely, to the use of concentrated solutions (a concentration ratio of 1:1 between yttrium and single rare earth elements) to evaluate the order of elution. To study the possibility of collecting the yttrium fraction separately and completely, a synthetic solution was prepared containing yttrium, rare earths, and the other major and minor elements commonly present in silicate rocks [19, 20]; Table 1 shows the ratios of the elements present in this solution and in the U.S.G.S. Standard granite G-2. After passage of small aliquots of solution through all the steps of the procedure, the yttrium fraction was collected and analyzed by the described method.

The results showed that the elution peaks are reasonably separated when yttrium and rare earth elements are present in the normal silicate ratio. The probable elution diagram is shown in Fig. 1b. For six aliquots of a solution

TABLE 1

Matrix element to yttrium ratios of a synthetic sample SS and the U.S.G.S. Standard granite G-2

Ratio	G-2	SS	Ratio	G-2	SS
Al_2O_3:Y	128,333	130,000	Ho:Y	0.03	0.1
Fe_2O_3:Y	2208	2500	La:Y	8	10
Na_2O:Y	3391	3500	Lu:Y	0.01	0.01
K_2O:Y	3758	3500	Nd:Y	5	5
MgO:Y	633	500	Pr:Y	1.58	2
CaO:Y	1617	1000	Sc:Y	0.31	0.5
TiO_2:Y	417	500	Sm:Y	0.61	0.5
Ba:Y	156	150	Sr:Y	40	50
Ce:Y	13	15	Tb:Y	0.05	0.01
Dy:Y	0.22	0.2	Th:Y	2.02	2
Er:Y	0.11	0.1	Tm:Y	0.03	0.01
Eu:Y	0.13	0.1	Yb:Y	0.07	0.1
Gd:Y	0.42	0.5	Zr:Y	25	25

TABLE 2

Averages and standard deviations of values for the yttrium content of some standard rocks and minerals

Sample	Average (ppm)	Standard deviation (ppm)	Value of Russell et al. [24]
NIM-G (granite)	110	25	130
NIM-S (syenite)	8	5	10
NIM-L (lujavrite)	35	8	30
NIM-N (norite)	5	3	7
NIM-P (pyroxenite)	5	3	—
NIM-D (dunite)	5	2	—

containing 1.56 ppm Y, 1.60, 1.62, 1.58, 1.48, 1.45, and 1.68 ppm Y were found. Also eight determinations were carried out on standard rock samples (NIMROC samples, South African Bureau of Standards). The results (Table 2) are generally within the ranges reported by Russell et al. [24], which indicates the utility of the method for the determination of yttrium in silicates.

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Short Communication

SEPARATION OF BENZIMIDAZOLE ANTHELMINTICS BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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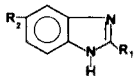
Since the demonstration of the anthelmintic properties of thiabendazole (TBZ) [1], many other compounds of the benzimidazole type have been synthesized and used in veterinary therapeutics: cambendazole (CBZ), mebendazole (MBZ), oxibendazole (OBZ), parbendazole (PBZ) and fenbendazole (FBZ). Of these, analysis for only TBZ has been undertaken so far. Various methods e.g. u.v. and i.r. spectrophotometry, fluorimetry and colorimetry have been used but the analysis is difficult and even gas chromatography has failed to give good accuracy and sensitivity [2, 3]. Tanaka and Fujimoto [4] succeeded in quantifying low levels of TBZ by gas chromatography after methylation but this is time consuming. More recently, Farrow et al. [5] have determined TBZ in the presence of post-harvest fungicides by h.p.l.c.



	R_1	R_2
Thiabendazole		H
Cambendazole		$(CH_3)_2CHOCONH-$
Mebendazole	$-NHCOOCH_3$	C_6H_5CO-
Oxybendazole	$-NHCOOCH_3$	C_3H_7O-
Parbendazole	$-NHCOOCH_3$	C_4H_9-
Fenbendazole	$-NHCOOCH_3$	C_6H_5S-

A simple, rapid method of analysis was required to detect the presence of any of these benzimidazoles in veterinary medicines. This report describes a method for the separation of six anthelmintic benzimidazoles by h.p.l.c. with two different chromatographic systems. The first involves a reverse-phase gradient system; the second involves normal partition chromatography with isocratic elution.

Experimental

Chromatographic system. A Varian LC 8500 high-pressure liquid chromatograph with u.v. detector operating at 254 nm and with a gradient system was used. The two chromatographic columns were a Lichrosorb RP8 (Merck) column (15 cm × 4.7 mm i.d.), particle size 10 μm, and a Lichrosorb NH_2 (Merck) column (15 cm × 4.7 mm i.d.), particle size 10 μm. In each case, the flow rate was maintained at 80 ml h^{-1}. The column pressure was 500 psi.

Chemicals. The reference samples of benzimidazoles were generous gifts from: Merck, Sharp and Dohme (TBZ and CBZ); Hoechst AG, Frankfurt/Main (FBZ); Lebrun SA, Paris (MBZ); Smith, Kline and French, Paris (OBZ); Distrivet, Paris (PBZ). All the solvents were analytical-reagent grade.

Analytical method. Three different mixtures were used. The benzimidazoles were dissolved in pure formic acid (5 ml) then in concentrated hydrochloric acid (5 ml) and in 50% (v/v) aqueous ethanol (90 ml). The mixture compositions were:

Solution A: TBZ 0.8 g l^{-1}, CBZ 0.12 g l^{-1}, MBZ 0.12 g l^{-1}, PBZ 2 g l^{-1}, FBZ 0.4 g l^{-1}.

Solution B: TBZ 0.8 g l^{-1}, CBZ 0.12 g l^{-1}, OBZ 0.4 g l^{-1}, PBZ 2 g l^{-1}, FBZ 0.4 g l^{-1}.

Solution C: OBZ 0.1 g l^{-1}, MBZ 0.1 g l^{-1}.

Solutions A and B were separated by reverse-phase gradient elution with the solvent: x% acetonitrile—aqueous 1% sulfuric acid with the program: 10% acetonitrile for 2 min then from 10% acetonitrile to 30% at the rate of 5% min^{-1} (Fig. 1A and B). OBZ and MBZ (Solution C) were eluted isocratically on an amino phase chemically bonded to silica gel (type NH_2) with the solvent: methylene chloride + isopropyl alcohol (90 + 10) — acetonitrile (50:50) (Fig. 1C). Initially, each of the benzimidazoles was separately chromatographed to determine their retention time and order of elution in these chromatographic systems.

Results and discussion

Figure 1 (A and B) shows typical chromatograms of solutions A and B. For solution A, the order of elution is TBZ — CBZ — MBZ — PBZ and FBZ. With the same column and the same solvent system, separation of OBZ and MBZ was not possible. A very slow gradient program (from 0 to 30% acetonitrile at the rate of 0.5% min^{-1}) allowed the initiation of a separation after 45 min. OBZ and MBZ have very similar chromatographic properties and consequently the same retention time on the RP8 column as is shown by the chromatogram of solution B, for which the order of elution is TBZ — CBZ — OBZ — PBZ and FBZ.

It was therefore necessary to use another column — an amino phase chemically bonded to silica gel (type NH_2). Figure 1C shows the separation of OBZ and MBZ in that order of elution. Separation is complete in about 5 min. This method of separation of benzimidazoles allows their presence in anthelmintic veterinary preparations to be detected.

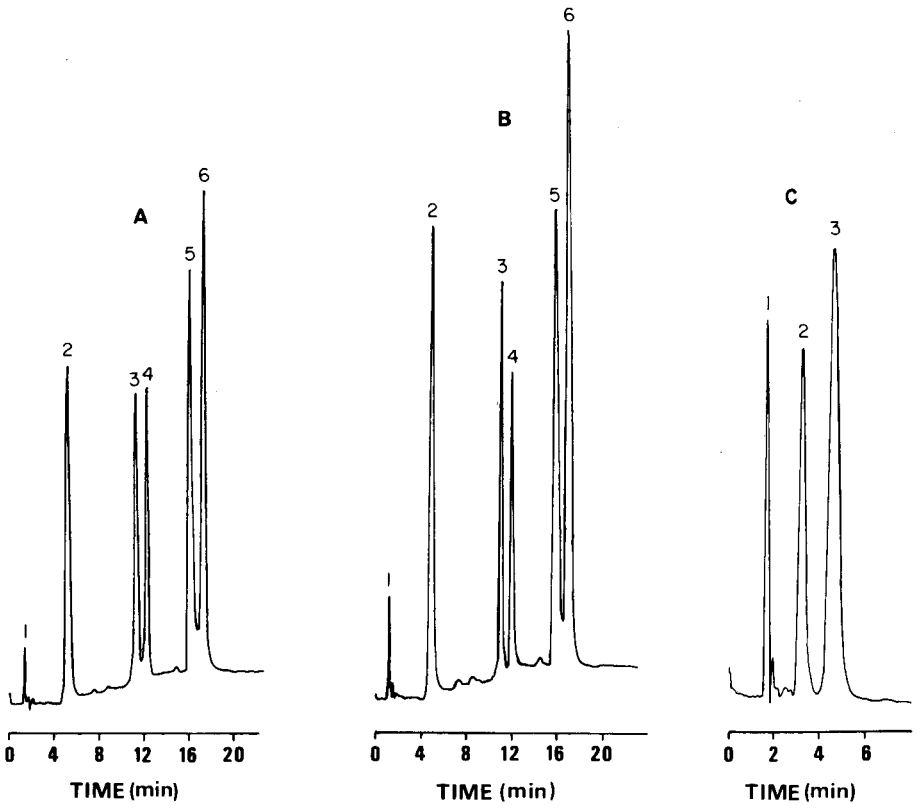


Fig. 1. Chromatograms showing the separation of benzimidazoles. (A) and (B) On a Lichrosorb RP8 microparticulate column with gradient elution (initial solvent composition, acetonitrile—aqueous 1% sulfuric acid (10:90) for 2 min; final solvent composition, acetonitrile—aqueous 1% sulfuric acid in water (30:70)); gradient rate 5% min^{-1}; flow rate 80 ml h^{-1}; detector sensitivity, 1.0 AUFS. A: 1 = injection artifact; 2 = TBZ; 3 = CBZ; 4 = MBZ; 5 = PBZ; 6 = FBZ. B: 1 = injection artifact; 2 = TBZ; 3 = CBZ; 4 = OBZ; 5 = PBZ; 6 = FBZ.

(C) On a Lichrosorb NH_2 microparticulate column with isocratic elution. Solvent composition: methylene chloride—isopropyl alcohol (90:10)—acetonitrile (50:50); flow rate 80 ml h^{-1}; detector sensitivity 0.5 AUFS. 1 = injection artifact; 2 = OBZ; 3 = MBZ.

Studies of the quantification of any of these benzimidazoles in veterinary medicines, after their detection by these chromatographic systems are being carried out with an internal standard. This method would be useful in the quality control of raw materials, in the quantification of benzimidazoles in foodstuffs of animal origin, and in the detection of residues. Sensitivity could be improved with a variable-wavelength u.v. detector operating at the maximum absorption (ca. 300 nm) of the benzimidazole concerned.

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Short Communication

A REACTIVATION SOLUTION FOR A COPPERIZED CADMIUM COLUMN IN THE AUTOMATIC DETERMINATION OF NITRATE IN NATURAL WATERS

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An accepted procedure for the determination of low levels of nitrate in natural waters involves a copperized cadmium column to reduce nitrate to nitrite, which is usually reacted with sulfanilamide under acidic conditions to form a diazo compound. The compound is then coupled with *N*-(1-naphthyl)-ethylenediamine to produce a reddish purple azo dye and determined colorimetrically [1, 2].

The automatic version of this method is now widely used [3, 4]. A simple reduction column consisting of a 1-m length of copperized cadmium wire threaded through a Teflon tube has been suggested [5], but granular cadmium is still commonly used, although regeneration of these columns can cause problems. This communication gives a method of maintaining reduction efficiency and prolonging the life of the reductor by pumping a reactivation solution through the reductor.

Experimental

Apparatus. A Technicon AutoAnalyzer II was used. Figure 1 shows the flow system. After debubbling, a three-way valve (Pharmacia) was inserted: one way flows through a copperized cadmium column for the reduction of nitrate to nitrite; by switching the valve, the other flows directly for the determination of nitrite. Nitrite, and nitrite plus nitrate, can thus be measured, without disconnection of the reduction column.

Reagents. Reagents were as for the Technicon Method [4].

Preparation of column. Granular cadmium (99.9%) in the form of nearly uniform cubes (0.7—1.2 mm; Wako, Osaka, Japan) was used. Copperized cadmium columns were prepared in two different ways. (a) The method of Wood et al. [2] without the use of a special washing bottle was applied. (b) Granular cadmium (ca. 10 g) was washed with 2 M hydrochloric acid in an Erlenmeyer flask, rinsed thoroughly with distilled water, washed with 0.3 M nitric acid, and rinsed with distilled water. The granular cadmium, treated with 50 ml of the reactivation solution (see below) for at least 24 h, was

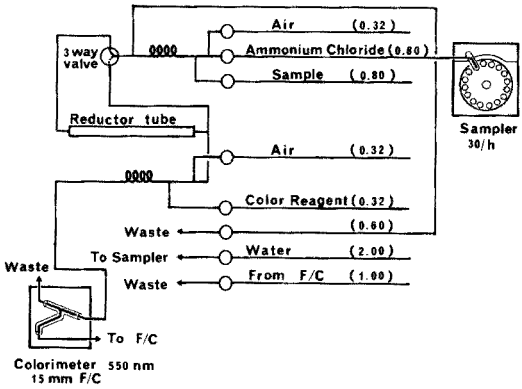

Fig. 1. Nitrate—nitrite manifold with a three-way valve. (Figures in parentheses signify flow rates in ml min^{-1}.)

covered with copper without the production of an excess of reduced copper particles. The prepared cadmium granules were transferred to a column tube, filled with water. Special precautions were not taken to avoid exposure of the prepared granules to air.

Reactivation solution. EDTA, disodium salt (38 g) was dissolved in ca. 700 ml of distilled water, and 12.5 g of copper sulfate was added. The pH was adjusted (2 M sodium hydroxide) to 7.0 and diluted to 1 l. This solution contains a 2:1 EDTA—copper(II) ratio.

Results and discussion

The main causes [6] of gradual decrease in efficiency are the decreased surface area of the reductor and the precipitation of hydroxides on the cadmium. The production of cadmium ions during the reduction of dissolved oxygen in water in the reductor is much larger than that caused by the reduction of nitrate [6], but the reduction of dissolved oxygen also produces copper ions, as copper from the top of the column gradually disappears during the analysis of fresh-water samples. This loss of copper probably causes the decreased reduction efficiency, and therefore the copper should be replaced at appropriate intervals to keep the efficiency constant. A reactivation solution should have the following properties: (1) copper should be deposited on the granular cadmium under continuous flow conditions without the production of copper particles; (2) the solution should remove precipitates of compounds of cadmium and other metals from the surface of the copperized cadmium.

Although the copper—EDTA complex is quite stable at pH 7—12, reduction of the chelated copper occurs on the surface of cadmium and consequently copper is deposited without the production of an excess of reduced copper particles. To take advantage of the solubility of cadmium hydroxide, the copper—EDTA complex solution at pH 7.0 is pumped through the system to reactivate the reduction column in situ.

A column which showed ca. 15% decrease in reduction efficiency could be reactivated by pumping the copper—EDTA solution through it for 5 min. When the reactivation solution was pumped through a copperized cadmium column in good condition, it was possible to shorten the conditioning time before analysis to ca. 15 min. When constant reduction of nitrate to nitrite was obtained, the reduction efficiency was always better than 99%.

As a decrease in the reduction efficiency can occur at any time, tedious checks on the reduction efficiency are necessary at frequent intervals. If the reactivation solution is passed through the reduction column after every 6 samples, the reduction column remains efficient for several months.

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Short Communication

4-NITRO-1,2-DIAMINOBENZENE AS A NEW CHROMOGEN IN THE WEST—GAEKE METHOD FOR SULFUR DIOXIDE

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The West—Gaeke method [1, 2] for the determination of sulfur dioxide in air is an established method and one to which other methods are frequently compared. In this method, nearly decolorized triprotonated pararosaniline is reconverted to a colored dye by reaction with hydrogensulfite released from a tetrachloromercurate(II) absorbing solution, and formaldehyde. The formation of a substituted monoalkylamine group, $-NHCH_2SO_3H$, from one of the $-NH_3^+$ groups on the protonated pararosaniline re-introduces resonance, as the reconstituted chromophore (λ_{max} = 560 nm) has nearly the same absorption spectrum as pararosaniline (λ_{max} = 543 nm). As the protonated pararosaniline reagent is present in excess, reaction of only one amino group per dye cation would appear to be favored in aqueous solution [3]. The reaction is versatile, having been used for the determination of formaldehyde [4] as well as sulfur dioxide and serving as the basis for the indirect determination of ozone [5] and sulfate aerosols [6].

Two reagents simpler in structure than pararosaniline have been reported: *p*-aminoazobenzene [7] and *p*-nitroaniline [8]. *p*-Aminoazobenzene, which absorbs at 386 nm in ethanolic solution [9], absorbs at 505 nm after reaction with formaldehyde and hydrogensulfite in aqueous 0.61 M hydrochloric acid solution. The absorption peak of *p*-nitroaniline at 387 nm is reduced in aqueous 1.6 M hydrochloric acid solution but regenerated on reaction with formaldehyde and hydrogensulfite. All three reagents — pararosaniline, *p*-aminoazobenzene and *p*-nitroaniline — give appreciable blanks which must be subtracted from absorbance values under the conditions of the methods reported.

4-Nitro-1,2-diaminobenzene was observed to be nearly colorless when protonated and red-orange when alkylated by the formaldehyde—hydrogensulfite process. This suggested possible utility as a chromogen in the West—Gaeke method. 3-Nitro-1,4-diaminobenzene, which is discussed along with 4-nitro-1,2-diaminobenzene as a donor-acceptor chromogen by Griffiths [10], produces a chromophore that absorbs at longer wavelengths but is less desirable as a reagent because of an appreciable blank. The absorption

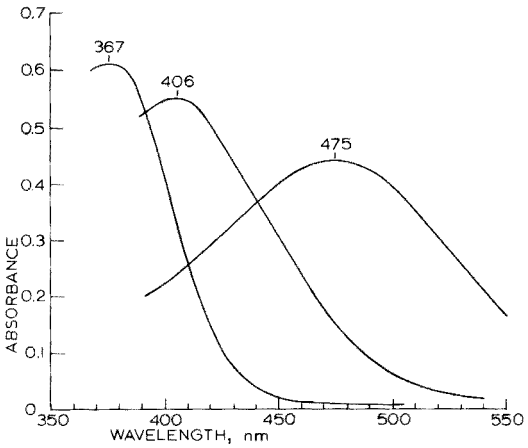

Fig. 1. Absorbance curves for 10^{-4} M 4-nitro-1,2-diaminobenzene: 406-nm peak, unprotonated in aqueous solution; 367-nm, protonated species in hydrochloric acid solution; 475-nm peak, alkylated species from reaction of formaldehyde and hydrogensulfite in hydrochloric acid solution.

maximum of 4-nitro-1,2-diaminobenzene in aqueous solution occurs at 406 nm, shifting to 367 nm in acidic solution (Fig. 1). The colored product formed on reaction with formaldehyde and hydrogensulfite absorbs at 475 nm. The blank is reduced to essentially zero when tetrachloromercurate anion, $[HgCl_4]^{2-}$, is present in hydrochloric acid solution of pH 0.4. The large bathochromic shift and the excellent blank both recommend 4-nitro-1,2-diaminobenzene as a reagent in the West–Gaeke method.

Experimental

Deionized water was used for preparing all reagent solutions and for making dilutions. Chemicals used were reagent grade or the purest grade available.

Reagent solution. Dissolve 27.2 g (0.1 mol) of mercury(II) chloride in several hundred ml of water and add 33.3 ml of concentrated hydrochloric acid. Dilute to 1 l, saturate with 4-nitro-1,2-diaminobenzene by stirring at room temperature for 1 h, and filter. The solution is stable but should be protected from strong light.

Formaldehyde solution. Dilute 1.0 ml of approximately 37% reagent-grade formaldehyde solution to 500 ml.

Hydrogensulfite stock solution. Prepare a 1,000 ppm hydrogensulfite stock solution by dissolving 1.28 g of sodium hydrogensulfite in water and diluting to 1 l. Prepare solutions of lesser concentrations by appropriate dilution. All these should be prepared fresh daily as needed.

Calibration curve. To 10.0 ml of reagent, add 1.0 ml of the formaldehyde solution and 1.0 ml of the appropriate hydrogensulfite solution. The pH of the resulting solution should be 0.4. Measure the absorbance in a 10-mm

TABLE 1

Data for calibration curve at 25°C

SO_2 (μg)	85	128	171	256
Absorbance	0.172	0.278	0.353	0.505
S.d. (n = 5)	0.020	0.035	0.027	0.021

cuvette at 475 nm after 35 min. The calibration curve data are shown in Table 1.

Interferences. As in previous similar methods, nitrogen dioxide, which decolorizes the reagent, can be tolerated in low concentrations that would not deplete the reagent. Hydrogen sulfide precipitates mercury(II) sulfide, which must be removed by filtration or centrifugation.

Results and discussion

The optimum pH and concentration of formaldehyde were found by varying each in turn. Above the concentration range 85—200 μg of sulfur dioxide, the calibration curve departs from linearity, probably because of depletion of the reagent. The linear relationship is approximated by the equation $\mu g\ SO_2 = 483A$, where A is absorbance. The faint blank observed for the 475-nm absorption peak (Fig. 1) for acidified solutions of 4-nitro-1,2-diaminobenzene disappears in the presence of tetrachloromercurate(II). The absorption peak at 475 nm is unaffected by the presence of tetrachloromercurate(II).

The large bathochromic shift in absorption maximum from 367 nm in the protonated reagent to 475 nm in the reaction product with formaldehyde and hydrogensulfite is comparable in magnitude to that exhibited by *p*-nitroaniline [8]. However, in the case of *p*-nitroaniline, the protonated species ($\lambda_{max} \approx 270$ nm) reacts with formaldehyde and hydrogensulfite to form a compound that absorbs at nearly the same wavelength as the unprotonated reagent ($\lambda_{max} = 387$ nm). The 387-nm peak persists in the spectrum of the protonated reagent and produces a sizeable blank. For the *p*-aminoazobenzene reagent, the absorption maximum of the protonated species has not been specified but it should be no greater than 386 nm [9]. The bathochromic shift from protonated reagent to reaction product would therefore be greater than in the case of 4-nitro-1,2-diaminobenzene, but a blank correction must still be made for the *p*-aminoazobenzene reagent.

The colored compound from the 4-nitro-1,2-diaminobenzene reagent is probably alkylated at the 1-amino position by a methylsulfonate group, if greatest interaction between an alkylamino donor group and the nitro acceptor group is to be assumed. Alkylation of an amino group in this type of chromophore customarily produces a bathochromic shift, but the shift observed for this reagent is surprisingly large and might be attributed to involvement of the 2-amino group in the resonance structures.

The principal advantage of 4-nitro-1,2-diaminobenzene as a reagent is the absence of a blank correction and its compatibility with acidified tetrachloromercurate(II) solution. Its lower solubility at the optimum pH for the reaction, which is reflected in the decreased range of linearity for absorbance vs. concentration, is a disadvantage.

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Short Communication

FLUORIMETRIC DETERMINATION OF LACTATE WITH IMMOBILIZED LACTATE DEHYDROGENASE

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Today most methods for the determination of lactate utilize the enzyme-catalyzed reaction

$$\text{lactate} + \text{NAD}^+ \overset{\text{LDH}}{\rightleftharpoons} \text{pyruvate} + \text{NADH}^+ + \text{H}^+$$

which has an equilibrium constant $K = 2.9 \times 10^{-12}$ mol l^{-1} at 25°C. By using a suitable buffer, the equilibrium can be displaced to the right [1, 2]. Most commonly, the increase in absorptivity at 340 nm caused by increasing concentrations of NADH is measured. Monitoring of the fluorescence intensity of NADH has also been successfully utilized. Electrochemical analysis based on entrapped or immobilized enzyme has been reported [4—6]. This communication describes a fluorimetric technique in which lactate dehydrogenase (LDH) is immobilized on sepharose to determine lactate.

Experimental

Apparatus. The apparatus included an Aminco-Bowman spectrofluorimeter with Xe—Hg lamp, disposable 1-ml syringes (Jintan Terumo Co. Ltd) and filters of pore size 1.2 μm (Millipore). The filter holders (Millipore Swinnex 13) were modified before use. A syringe with filter holder is shown in Fig. 1.

Reagents. Lactate dehydrogenase type XI (870 U mg^{-1} from rabbit muscle), nicotinamide adenine dinucleotide (NAD; grade III from yeast), and crystalline lactic acid (grade L-VII) were purchased from Sigma. CNBr-activated Sepharose 4B was obtained from Pharmacia.

Preparation of immobilized enzyme. Lactate dehydrogenase was immobilized on CNBr-activated Sepharose 4B as described by Axén et al. [7]. The resulting gel was packed into the filter holders which were then filled with 2 M ammonium sulfate (containing ca. 1% thymol to prevent bacterial growth). The filter holders were stored in a refrigerator.

Procedure. In a 10-ml beaker, 0.5 ml of glycine buffer [2] was mixed with 0.25 ml of NAD solution (3 mg ml^{-1}) and 0.25 ml of sample. The mixture was drawn into the syringe, which was then placed on the filter

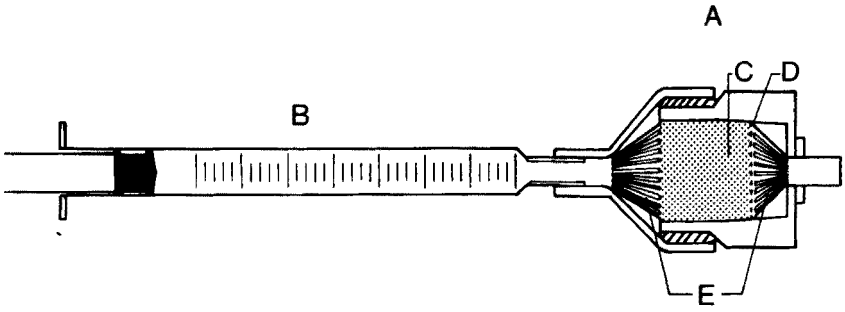

Fig. 1. A filter holder (A) adapted with a disposable syringe (B). The gel with the immobilized enzyme (C) and filter (D) are inserted between two porous plugs (E) in the filter holder.

holder supporting the gel; 0.5 ml of the mixture was immediately pushed into the filter holder. The solution was incubated for 1 min in the filter holder, after which 0.2 ml of the solution in the filter holder was discarded and the subsequent 0.2 ml was diluted with 1 ml of triply-distilled water. The fluorescence was measured on this diluted sample (λ_{em} = 459 nm).

The filter apparatus was flushed with 5 ml of glycine buffer between each measurement. Standards in the range 1×10^{-4} M—1×10^{-2} M lactate were made by diluting a weighed amount of lactic acid with triply-distilled water. When a stored filter holder was first put into use, it was activated with 1×10^{-2} M lactic acid for 45 min.

Results and discussion

Fluorescence intensity as a function of the incubation time is shown in Fig. 2. An incubation time of 1 min was considered convenient with regard to the speed of analysis. The useful range was 1×10^{-4}—1×10^{-2} M lactate, but the sensitivity could be raised by using more immobilized enzyme and/or a longer incubation period. A typical calibration curve is shown in Fig. 3. Seven consecutive measurements of the same sample gave a mean fluorescence intensity of 109 arbitrary units with a range of 101—106 units and a standard deviation of 6.2 units. More than 40 determinations were made during 14 days with the same preparation; the immobilized enzyme still retained enough activity to be used in the same sensitivity range as at the beginning.

Storage of unused filter holders with enzyme had no noticeable effect on the sensitivity. Lactate dehydrogenase immobilized in a polyacrylamide matrix was also tested but this preparation retained sufficient activity for only six days of regular use.

The normal lactate concentration in human blood is about 1 mM, which means that this method is useful for analysis of blood samples (the serum proteins must be removed before the analysis). In comparison to other published methods, the immobilized lactate dehydrogenase technique described here is easily adaptable to routine analysis. A filter fluorimeter would be sufficient for this application.

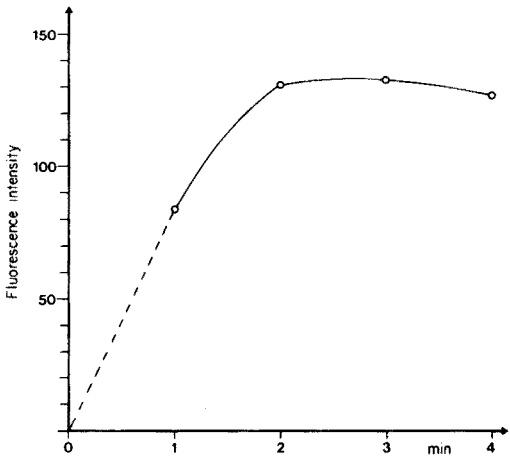

Fig. 2. Fluorescence intensity (in arbitrary units) as a function of the incubation time.

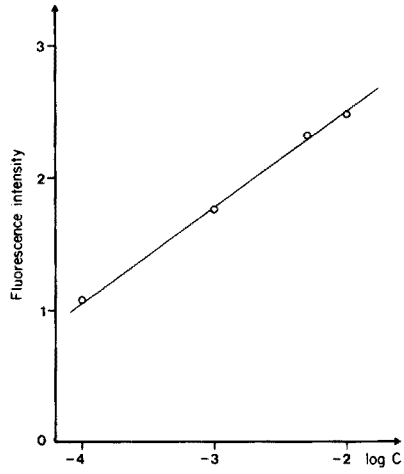

Fig. 3. Fluorescence intensity (in arbitrary log units) as a function of the lactate concentration (in log M units). All the measurements were made in duplicate.

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Short Communication

A TITRIMETRIC METHOD FOR DETERMINATION OF FREE FATTY ACIDS IN TISSUES AND LIPIDS WITH TERNARY SOLVENTS AND *m*-CRESOL PURPLE INDICATOR

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Since a method for the estimation of free fatty acids (FFA) was reported by Ayers in 1956 [1], more than 20 papers have given modifications of her procedure and applications to various samples. Recently, photometric [2—4] and enzymatic [5] methods have been developed for estimating the total FFA in biological samples. Titrimetric determination, however, is still widely employed for quality assurance and characterization of product degradation in the food and edible oil industries [6].

The content of FFA in fish tissue and lipids has been used as a routine quality index for fish products [7, 8]. The American Oil Chemists' Society method [9] for determining FFA is unsatisfactory for fish oils that are yellow or red from carotenoids, or brownish from poor quality [10], because the phenolphthalein end-point is masked. Despite special precautions, such as multiple determinations, great difficulty may still be experienced in obtaining consistent and accurate values for FFA in some fish lipid samples with the AOCS or similar methods [6, 10, 11]. Bligh and Scott [12] used sodium methylate with an automatic titrator to determine FFA in chloroform-methanol extracts of fish tissues, but a simpler procedure would be an advantage; aqueous NaOH is more convenient than an alcoholic solution.

An improved titrimetric method for determining the FFA content in various lipids, even when the samples are highly coloured or rancid, is described here. A ternary solvent system and *m*-cresol purple indicator are used, and the method can be applied directly to the determination of total FFA in extracts of fish tissue samples without isolating the lipids completely.

Experimental

Reagents. An aqueous solution (0.5%) of *m*-cresol purple (Fisher Scientific Co.) was used as the indicator. Phenolphthalein, 1% in ethanol (95%), was used for comparative titrations with aqueous NaOH (0.05 and 0.10 M). All other chemicals were ACS grade. Lipid samples were extracted by the method of Bligh and Dyer [13].

Transfer of lipids from tissue to chloroform. Part of the extraction procedure of Bligh and Dyer [13] was used for preparing the tissue sample for the FFA determination. A tissue sample (10—20 g ± 0.01 g) was weighed out in a 200-ml Vir-tis homogenizing flask. After 50 ml of chloroform, 50 ml of methanol and 40 ml of distilled water had been added, the tissue sample and solvents were blended vigorously for at least 3 min in a Vir-tis Model 23 homogenizer. The homogenized mixture was filtered under vacuum through Whatman No. 1 filter paper on a Buchner funnel (85 mm o.d.). The filtrate was transferred to a 250-ml separatory funnel; the chloroform extract (ca. 50 ml, bottom layer) was separated and dried by passing it through a fluted filter paper, containing 10 g of anhydrous Na_2SO_4, into a 250-ml Erlenmeyer flask suitable for titration of the FFA content. Alternatively, the scale of extraction can be increased (say 5-fold), the chloroform layer separated and made up to a convenient volume with a chloroform—methanol mixture (95:5); aliquots are then used for titration.

Preparation of lipid samples. The lipid sample (ca. 1 ± 0.005 g) was accurately weighed in a small sample vessel and dropped into a 250-ml Erlenmeyer flask containing 50-ml of chloroform. This solution was used for the following titration.

Titration for FFA. A ternary solvent system was prepared by adding 25 ml of methanol and 50 ml of isopropanol to 50 ml of the chloroform sample solution. After 5 drops of *m*-cresol purple indicator had been added, the solution was titrated to the purple end-point with standard aqueous 0.05 M NaOH solution; magnetic stirring was used. A blank titration, (usually about 0.1 ml of 0.05 M NaOH) should be made daily.

Calculation. The FFA may be calculated as FFA (μmol g^{-1} of sample) = 1000 NV/W or as FFA (% of sample, as oleic acid) = 28.2 × NV/W where N, V, and W denote the molarity of the titrant, the volume (ml) of titrant, and weight (g) of sample, respectively. FFA, calculated as % oleic acid in the sample, has been widely used for quality control of foodstuffs [7, 8]. The proportions of the various fatty acids vary greatly in different lipids and foodstuffs [14, 15], however, and a molar unit, such as μmol g^{-1} tissue, should be used to express the FFA content as correlations with other data are more easily made.

Results and discussion

Determination of FFA in tissue and lipid samples. The FFA value was determined in fish tissues by transfer into a chloroform layer without prior isolation of the lipids; the values obtained, in weight percentage and molar units, are compared in Table 1.

Lipid samples were redissolved and the FFA contents determined by titration with the solvent—indicator system described above; at concentrations of FFA up to 13%, the results obtained were slightly higher than those obtained with the AOCS procedure, but had a much lower standard deviation (Table 2). Reproducibility was satisfactory, even for highly-coloured

TABLE 1

Determination of FFA in fish tissues by the direct titrimetric method

Sample	FFA content in tissue		
	As oleic acid, (%)	μmol g^{-1}	R.s.d.[a] (%)
Cod fillet	0.045	1.6	4.8
Mackerel white meat	0.322	11.4	5.2
Minced capelin	0.409	14.5	2.0
Herring fillet	0.567	20.1	3.0
Fish meal	1.07	38.0	2.8
Mackerel dark meat	1.27	45.1	1.5
Cod liver	1.72	60.9	4.1
Mackerel skin	2.70	95.6	1.9

[a]Calculated from 7 determinations.

TABLE 2

Comparison of the FFA values for marine lipids given by the proposed method and the AOCS method

Lipid sample	Oleic acid added (%)	AOCS method % FFA ± s.d.[a]	This method % FFA ± s.d.[a]
Herring oil	—	1.58 ± 0.06	1.60 ± 0.02
Herring oil	4.59	5.05 ± 0.23	5.13 ± 0.04
Redfish oil	—	2.87 ± 0.11	2.92 ± 0.02
Redfish oil	12.49	15.20 ± 0.38	15.52 ± 0.04
Oxidized herring oil	—	2.58 ± 0.17	2.63 ± 0.04
Oxidized herring oil	10.22	12.38 ± 0.57	12.85 ± 0.11
Oxidized mackerel oil	—	8.41 ± 0.59	8.60 ± 0.14
Oxidized redfish oil	—	5.01 ± 0.28	4.95 ± 0.07
Cod frame oil	—	—	1.98 ± 0.02
Mackerel oil	—	—	2.70 ± 0.03
Cod liver oil	—	—	3.34 ± 0.02
Mackerel frame oil	—	—	4.81 ± 0.07
Seal oil	—	—	5.26 ± 0.03

[a]Calculated from 7 determinations.

oils from oxidized flesh and from liver, when *m*-cresol purple indicator was used. Quantitative recoveries of oleic acid added to three of the lipid samples were given by both methods.

Selection of solvent and indicator. The cloud-point method [16] was used to measure the solubility of water in various solvent systems. The chloroform—methanol—isopropanol system (2:1:2, v/v) could be mixed with ca. 16% of its volume of aqueous NaOH before becoming turbid. The colour change of *m*-cresol purple (yellow to purple at pH 7.4—9) was much more

TABLE 3

Recovery by the proposed method of various FFA added to cod fillet tissue

Fatty acid[a]	FFA added[b] (μ-mol g^{-1} tissue)	Recovery[c] (%)	Fatty acid[a]	FFA added[b] (μ-mol g^{-1} tissue)	Recovery (%)
C 2:0	127	16	C 6:1	50	74
C 4:0	71	65	C 8:1	61	87
C 6:0	61	76	C 10:1	54	94
C 8:0	69	89	C 14:1	30	98
C 10:0	62	95	C 16:1	16	101
C 12:0	68	98	C 18:1	38	99
C 14:0	54	102		115	100
C 16:0	60	100	C 18:3	30	99
C 18:0	50	98	C 18:4	16	101
	140	102	C 20:5	14	101
			C 22:6	11	99

[a]CX: n denotes a fatty acid of X carbons in a straight chain, with n double bonds.
[b]20 g of tissue used for all determinations.
[c]% recovery calculated from 4 determinations, with a relative deviation less than 2%, for the fatty acids >C8.

readily detectable, in the presence of the colours frequently found in extracts of marine lipids and foodstuffs, than that of phenolphthalein or alkali blue.

Recovery of fatty acids. Fatty acids were added to samples of good-quality cod fillet low in FFA; the mixtures were extracted and titrated by the ternary solvent—*m*-cresol purple procedure (Table 3). The fatty acids with chains longer than C10 gave consistently good recoveries with relative errors of ca. 2%. However, the percentage recovery decreased proportionately with the chain length, from 96% recovery for C10 acids to 16% for acetic acid, and the recoveries of all monoenes were slightly less than those of the corresponding saturated acids. Long-chain polyenes, e.g. 18:4, 20:5, and 22:6, were extracted and determined as efficiently as other fatty acids. Since less than 2% of the short-chain fatty acids (<C10) occur in most food tissues and lipids [17], the method described meets the requirements for the determination of FFA in various food and oil samples.

Conclusions

The titrimetric method described has advantages over the AOCS procedure, in terms of greater reproducibility, sharper end-point, smaller sample size, superior miscibility of titration mixture, and direct application to various biological samples. It can be employed for the determination of the FFA content of fats and oils, particularly in highly coloured and oxidized samples. For tissue and other biological samples, the method gives a direct estimation of FFA without the extraction of lipids.

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Short Communication

THE STABILITY SEQUENCE OF THE TRIVALENT RARE EARTH CHELATES OF 2-NITROSO-1-NAPHTHOL-8-SULPHONIC ACID IN AQUEOUS SOLUTION

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The stability sequence in the series of lanthanide complexes formed with 2-nitroso-1-naphtholsulphonates has been studied previously by the customary potentiometric and spectrophotometric methods [1]. In the present work a new method was used to determine the stability order of the primary lanthanide complexes formed with 2-nitroso-1-naphthol-8-sulphonic acid. The acid was titrated spectrophotometrically with sodium hydroxide in the presence of more than ten-fold excess of each of the lanthanides in aqueous potassium chloride solutions at 25°C. The values obtained here add to the list of known stability constants of the lanthanide chelates of nitrosonaphthols.

Experimental

Reagents. Sodium 2-nitroso-1-naphthol-8-sulphonate was prepared from 1-aminonaphthalene-8-sulphonic acid (K and K Laboratories, technical) [2]. Lanthanide(III) perchlorates were prepared from the corresponding oxides, except for the cerium(III) salt, which was cerium nitrate.

Apparatus and methods. Spectrophotometric titrations were carried out in a flat-bottomed glass cylinder (volume ca. 200 cm^3) immersed in a water thermostat (25.0 ± 0.1°C). Purified nitrogen gas was passed through the solutions during the titrations; magnetic stirring was also used.

A Perkin-Elmer Model 402 u.v. spectrophotometer connected to a digital voltmeter Mk III (Weiss Electronics Ltd.) was used for the spectrophotometric measurements. The solution was transported from the titration vessel to the flow-through cell of the spectrophotometer at a flow rate of about 20 ml min^{-1} by a Masterflex pump with Tygon tubes.

A Radiometer PHM 64 potentiometer equipped with a Beckman glass electrode and an open liquid junction saturated calomel reference electrode was used for the pH measurements during the titration. The hydrogen ion concentrations were calculated with the aid of the apparent activity coefficient values given by Näsänen et al. [3].

The formation constants of the primary metal chelates, $K_1 = [H^+][LnL^+]/[Ln^{3+}][HL^-]$, were determined under experimental conditions where the lanthanide was present in excess. It was assumed that no other chelates were

formed. The absorbances of the solutions to be titrated in the buffer range were measured at several wavelengths and the calculations were done with the equation

$$K_1 = \{(\epsilon - \epsilon_{HL^-})\,[H^+] + (\epsilon - \epsilon_{L^{2-}})\,K_a\}\,/(\epsilon_{LnL^+} - \epsilon)\,[Ln^{3+}] \qquad (1)$$

TABLE 1

The pK_1 value of the dysprosium chelate of 2-nitroso-1-naphthol-8-sulphonic acid calculated from spectrophotometric titration data. $c = 1.54 \times 10^{-4}$ M, $c_{Dy} = 2.02 \times 10^{-3}$ M, $I = 0.092$, 25°C

$-\log [H^+]$	455 nm	465 nm	475 nm	485 nm	495 nm	pK_1 (mean)
4.373	2.492	2.507	2.521	2.509	2.498	2.505
4.596	2.510	2.516	2.514	2.512	2.502	2.511
4.729	2.501	2.512	2.525	2.507	2.499	2.509
4.883	2.507	2.517	2.518	2.518	2.505	2.513
5.005	2.511	2.506	2.509	2.513	2.508	2.509
5.138	2.521	2.516	2.515	2.509	2.506	2.513
5.277	2.521	2.510	2.509	2.510	2.501	2.510
5.390	2.515	2.509	2.506	2.506	2.507	2.509
5.525	2.515	2.506	2.502	2.503	2.499	2.505
pK_1 (mean)	2.510	2.511	2.513	2.510	2.503	2.509

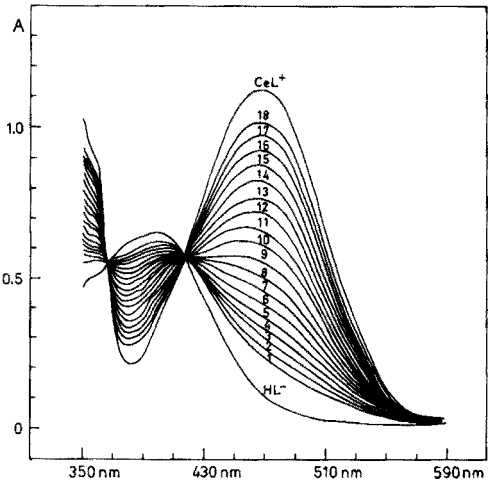

Fig. 1. Titration spectra of the system sodium 2-nitroso-1-naphthol-8-sulphonate and $Ce(NO_3)_3$. Curves 1 and 18 refer to solutions where $-\log [H^+] = 4.39$ and 6.42, respectively. $c = 1.54 \times 10^{-4}$ M, $c_{Ce} = 1.98 \times 10^{-3}$ M, $I = 0.052$, 25°C.

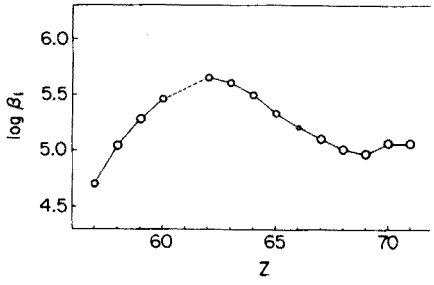

Fig. 2. Stability constants of the rare earth chelates versus atomic number at 25°C and $I = 0.1$. The radii of the circles are the errors given in Table 2.

The value of the naphtholic dissociation constant of the ligand needed in the calculations was obtained from the Debye—Hückel equation [2]:

$$pK_a = 8.19 + 0.30\,I - 2.036\,I^{\frac{1}{2}}/(1 + 1.05\,I^{\frac{1}{2}}) \quad (2)$$

(In these equations, all symbols have their conventional meanings.)

Results and discussion

The results of the spectrophotometric determination of the equilibrium constant of the primary dysprosium chelate are presented in Table 1. The spectra recorded at different stages of cerium chelate formation are shown in Fig. 1. The log β_1 values ($\beta_1 = K_1/K_a$) of the lanthanide and yttrium chelates at two different ionic strengths near 0.1, and the equilibrium and stability constants interpolated to ionic strength 0.1 are collected in Table 2. The known stability sequence of the rare earth chelates of this type of ligand was confirmed. The stability increases with increasing atomic number from lanthanum to samarium, decreases to thulium, and again increases slightly to lutetium (Fig. 2).

In general, 2-nitroso-1-naphthols form weaker metal complexes than do 1-nitroso-2-naphthols [1]. However, the rare earth complexes of 2-nitroso-1-naphthol-8-sulphonic acid, like those of 2-nitroso-1-naphthol-4,8-disulphonic-acid, which have the sulphonate group in the *peri*-position to the hydroxyl group, are very stable [4]. They are even stronger than any of the lanthanide chelates of 1-nitroso-2-naphtholsulphonates studied previously.

The equilibrium constants of the yttrium and cerium chelates at different

TABLE 2

The log β_1 values of the rare earth complexes of 2-nitroso-1-naphthol-8-sulphonic acid at two different ionic strengths, and the log β_1 and pK_1 values interpolated to ionic strength 0.1. Errors given are three times the standard deviations

M	I	log β_1	I	log β_1	log β_1 (I = 0.1)	pK_1 (I = 0.1)
La	0.0928	4.73	0.1128	4.65	4.70 ± 0.03	3.04
Ce	0.0919	5.07	0.1119	4.99	5.04 ± 0.04	2.70
Pr	0.0924	5.31	0.1125	5.23	5.28 ± 0.04	2.46
Nd	0.0924	5.49	0.1123	5.40	5.46 ± 0.03	2.28
Sm	0.0933	5.68	0.1134	5.58	5.65 ± 0.03	2.09
Eu	0.0920	5.65	0.1119	5.56	5.61 ± 0.03	2.13
Gd	0.0944	5.52	0.1144	5.44	5.50 ± 0.03	2.24
Tb	0.0919	5.37	0.1119	5.27	5.33 ± 0.03	2.41
Dy	0.0920	5.24	0.1120	5.15	5.21 ± 0.02	2.53
Ho	0.0918	5.13	0.1118	5.04	5.10 ± 0.04	2.64
Er	0.0918	5.03	0.1118	4.98	5.01 ± 0.04	2.73
Tm	0.0982	4.98	0.1181	4.91	4.97 ± 0.04	2.77
Yb	0.0920	5.09	0.1119	5.01	5.06 ± 0.04	2.68
Lu	0.0963	5.07	0.1163	4.96	5.06 ± 0.04	2.68
Y	0.0942	4.85	0.1142	4.75	4.82 ± 0.02	2.92

TABLE 3

Parameters pK_1^0 and α and formation constants of yttrium and cerium complexes of 2-nitroso-1-naphthol-8-sulphonic acid in solutions of various ionic strengths

Yttrium: α = 1.54			Cerium: α = 1.38		
I	pK_1 (obs.)	pK_1 (calc.)	I	pK_1 (obs.)	pK_1 (calc.)
0		2.053	0		1.784
0.0143	2.464	2.464	0.0118	2.135	2.169
0.0543	2.748	2.751	0.0521	2.503	2.491
0.0942	2.898	2.902	0.0919	2.675	2.654
0.1142	2.971	2.960	0.1119	2.729	2.715
0.2140	3.151	3.154	0.2121	2.933	2.929
			0.3121	3.050	3.067

ionic strengths are given in Table 3. The Debye—Hückel equation $pK_1 = pK_1^0 + 4.072\, I^{\frac{1}{2}}/(1 + \alpha\, I^{\frac{1}{2}})$, was used to express the dependence of pK_1 on ionic strength. The thermodynamic values of pK_1 and the values of the parameter α obtained by fitting this Debye—Hückel equation to the experimental data by the method of least squares are also presented in Table 3. As expected, the yttrium chelate is as stable as the chelates of the lightest lanthanides.

The complex formation between 2-nitroso-1-naphthol-8-sulphonic acid and zinc, cadmium, nickel and europium ions has been studied potentiometrically in this laboratory [2, 5, 6]. The value of the stability constant for EuL^+ obtained potentiometrically is slightly smaller than the value reported here. No other data for these lanthanide complexes seem to be available.

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Analytica Chimica Acta, 99 (1978) 397—398

A Critical Comment on: An Indirect Method for the Sequential Determination of Silicon and Phosphorus in Rock Analysis by Atomic Absorption Spectrometry

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(Received 29th November 1977)

The potential usefulness of the method proposed by Riddle and Turek [1] for the sequential determination of silicon and phosphorus in rock analysis must be questioned. Techniques for the analysis of silicate rocks have seen many changes in recent years. Most of the newer methods have involved improvements in speed or in precision and accuracy. Some appear more readily adaptable to use by relatively unskilled personnel than were the classical (largely gravimetric) procedures.

The determination of silica, a constituent normally present at the 50% level or higher, has been a persistent problem in all schemes of rock analysis. Few, if any, of the newer analytical systems have been able to surpass the reliability of a classical gravimetric determination in the hands of a skilled analyst. Nevertheless, many new approaches have been proposed, involving refined purely chemical procedures, spectrophotometry, atomic absorption, x-ray fluorescence and even direct-reading optical emission spectrometry. Generally, such methods have greatly improved productivity, and in some cases have approached the reliability of the classical procedure.

In the light of the above, one cannot but wonder at the method proposed by Riddle and Turek [1]. Consider the following points.

(a) Ten separate determinations on the same sample gave results ranging from 53.7 to 65.9% silica. The mean is close to the accepted value, but it is difficult to see how a geologist could use data produced by a method with a standard deviation of 3.3% silica — a coefficient of variation of over 5% at the 60% level. The range of phosphorus pentoxide results on the ten replicates of one sample is almost as great as can be observed on a wide variety of usual rock types; the coefficient of variation exceeds 50%.

(b) Involving, as it does, two separate solvent extractions, pH adjustments, atomic absorption measurements and application of corrections, the method is clearly very much slower than any of the known methods based on colorimetry or direct atomic absorption measurements, none of which produces results of such high variability.

(c) The method has been tested on only one actual sample, and that not a recognized international reference material. Many of the latter are now

available, covering a wide range of compositions. Tests on such real samples are more convincing than the use of pure solutions to establish limiting ratios.

(d) The statement (on p. 49) that "Wet chemical techniques, based on heteropolymolybdate formation, require the separation of P, Si and Ti from each other" is at variance with the large number of published methods for photometric determination of the two elements of interest.

Had the material been presented as a study of the chemistry of sequential determination of silicon and phosphorus via atomic absorption measurement, it might have been of some academic interest. However, the title specifically claims to describe a method for use in rock analysis. The authors' own reported data clearly indicate its unsuitability for that purpose.

REFERENCE

1 C. Riddle and A. Turek, Anal. Chim. Acta, 92 (1977) 49.

Reply to the Critical Comment by S. Abbey and J. A. Maxwell

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The paper criticized was presented as a study of the sequential determination of silicon and phosphorus via atomic absorption measurement. The rationale for the research was developed in the introduction. Possibly the results are only of academic interest. The subject investigated was the practicality of atomic absorption analysis of aqueous solutions, derived from organic extractant phases, as applied to silicon and phosphorus analysis of rock samples. From the data reported, any analyst may decide whether or not the method can be of use in his particular situation.

Abbey and Maxwell criticize the title of the original paper; however, the paper undeniably describes "an indirect method for the sequential determination of silicon and phosphorus in rock analysis by atomic absorption spectrometry". Nowhere was it claimed or implied that the method should necessarily replace existing methods. Had that been done, we should, in general, concur with the criticisms of Abbey and Maxwell.

Analytica Chimica Acta, 99 (1978) 399—403

Book Reviews

R. Belcher (Ed.), *Instrumental Organic Elemental Analysis*, Academic Press, London, 1977, ix + 299 pp., price £14.00, $27.35.

This 13th volume in the series on "The Analysis of Organic Materials" under the general editorship of R. Belcher and D. M. W. Anderson is superficially very different from the others in the series in at least two respects. First, it is a collection of six review chapters by different authors, in contrast to the more monographic nature of previous volumes. Secondly, and perhaps more significantly, it reviews the determination of various elements not so much by technique as by specific manufacturer's instruments, e.g. C, H and N; S and O; C, H, N, S and O. Also described are the determination of nitrogen by a modification of the Dumas method using another manufacturer's instrument, the microcoulometric determination of S, Cl, N and individual compounds by two other commercial instruments (but mainly one of them) and more broadly and in tune with previous volumes, the determination of 'organic' carbon in water by a range (8) of commercial instruments. Even in the last instance, however, the names and addresses of the manufacturers are given.

The emphasis on analysis by specific manufacturer's equipment does, at first sight, appear to be at odds with the general principles of scientific textbooks such as this. However, the days of skilled analysts and their special analytical knowledge and expertise have gone. They have now been replaced almost entirely by the inanimate self-correcting automaton fed by technicians and tended by technologists skilled in electronics and the like. Whilst this may be regarded as another instance in which chemists have engineered themselves out of jobs by their own skilled efforts, it has to be confessed that the routine drudgery has now been passed more to the technician than to the graduate chemist and that, on the whole, because of the automated control of experimental variables the results produced are likely to be more reproducible for routine analysis — though not necessarily more accurate.

Unquestionably the text gives a very good and full account of the operation of these specific instruments, though it would have been very welcome to have had more assessment of their limitations and their performance assessed against those of competitors in the market place. Perhaps others elsewhere are engaged on this very task. However, be that as it may, the present book should be of enormous help to all who are engaged in this work, particularly since no previous textbook has gone over automated elemental analyses. There seems little likelihood that the laboratory scene on automated C, H, N, etc., analyses will change markedly over the next decade, though no doubt the instrument manufacturers will add a few more refinements in

their continued efforts to improve their performance and saleability vis à vis their competitors. It is this feature that is most likely to "age" the present volume which otherwise should act as a reference work for these particular instruments for many years to come.

T. S. West

J. D. Winefordner (Ed.), *Trace Analysis: Spectroscopic Methods for Elements*, J. Wiley, New York, 1976, xii + 484 pp., price £18.00, $29.45.

The editor's aim is to foster critical comparisons of techniques of trace elemental analysis by using clearly defined numerical values for analytical and instrumental merit. The result is a unique blend of the practical aspects, chemical and physical principles which avoids the more common, instrument by instrument, semi-catalogue approach.

The first five chapters include general material concerning sources and propagation of errors, resolving power of optical spectroscopic methods, signal measurement, chemical aspects of elemental analysis, signal intensity expressions for a variety of atomic and molecular spectroscopies, characteristics of spectrometer and typical overall systems. The chapters which follow are devoted to techniques: atomic and molecular spectroscopy, fluorimetry, nuclear and x-ray methods, spark-source mass spectrometry, followed by a detailed and critical chapter on the comparison of the spectroscopic methods discussed earlier. A series of appendices deals with Kaiser's concept of informing power, sequential versus multichannel versus multiplex measurements and the bases of Fourier and Hadamard transform spectrometry. These appendices could with advantage have been expanded and promoted to chapter status.

The book will repay careful study by those who wish to select and by those who have no choice of technique or equipment but who need a frank appraisal of the overall position and a guide to optimization. Industrial and academic libraries, teachers and serious students af analytical spectroscopy should purchase and read this stimulating text.

D. Thorburn Burns

E. Wanninen (Ed.), *Essays on Analytical Chemistry* (in Memory of Professor Anders Ringbom), Pergamon Press, Oxford, 1977, xiv + 607 pp., price $50.

Memorial and honour volumes are rare tributes in the chemical world, and it is most appropriate that the significant scientific achievements of Anders Ringbom be acknowledged in this way for posterity. The volume contains 52 papers/essays reviewing the background to current developments as well

as surveying many areas of present research. The papers, from a world-wide authorship, are grouped under the following headings: chemical equilibria, titrations, photometric analysis, electrochemistry, separations, trace analysis, kinetic analysis and lastly other analytical topics. The length of sections and overall balance of material quite naturally reflects the research interests of the late Professor Ringbom. The first paper is by the doyen of the subject, Professor I. M. Kolthoff.

The book will be of interest to all analytical chemists, and should be regarded as compulsory reading for all postgraduate students; particularly the material under 'other analytical topics' on sampling, statistical analysis, development of techniques and literary communication will be helpful in their generating a professional approach to the subject.

D. Thorburn Burns

D. Purves, *Trace-element Contamination of the Environment*, Elsevier, Amsterdam, 1977, xi + 260 pp., price Dfl. 85, U.S. $34.75.

This is the first volume in a series entitled Fundamental Aspects of Pollution Control and Environmental Science, edited by R. J. Wakeman. The author has restricted himself to discussing the processes of trace-element dispersion in the biosphere and to highlighting only those aspects that appear to him to be of vital importance. More than half the book consequently concerns the Soil, with chapters on its trace-element composition and contamination, the availability of trace elements, and their effects on plants and animals. Contamination of the atmosphere and hydrosphere are also discussed, but rather briefly. The emphasis given to lead, cadmium and mercury in the first chapter, and to the disposal of municipal waste, wastewater and sewage sludge in the final chapter, clearly reflects the author's field of interest. Over 400 references are cited.

The author reports much of his own environmental work in detail, but the interpretation given is not always easy for the reader to understand. For example, although herbage lead contents are noted on page 152 to be significantly correlated with lead in soil extracts, despite the earlier warning on page 57 concerning exceptional values, the comment is made on page 153 that the high herbage contents may be attributed to foliar contamination. The association of weakly-linked facts such as the vapour pressure of mercury metal and the mouth temperature of dental amalgam, on page 26, is likely to mislead the unwary. However, if the book is read with care, it is a useful source of information on a topical subject, especially for those who are concerned with possible agricultural consequences of trace-element contamination.

J. C. Burridge

Stephen G. Schulman, *Fluorescence and Phosphorescence Spectroscopy: Physicochemical Principles and Practice*, Pergamon Press, Oxford, 1977, x + 288 pp., price $20.00.

This is volume 59 in the International Series in Analytical Chemistry edited by Professors Belcher and Freiser. In order to make the material available as economically and rapidly as possible, the author's typescript has been reproduced in its original form. A reasonably clear and uniform effect has been achieved; around a dozen typists' errors were noticed — the majority associated with the names of chemical compounds or experimental details such as 0.1 NH_2SO_4.

The author's intention was to write a book for analytical chemists and biological scientists and to make the instrumental, structural and environmental aspects of luminescence spectra intelligible to readers "with a general college background in chemistry and physics". In general this has been achieved. The theoretical and instrumental design and operating principles are explained clearly with the minimum of mathematics; there are comprehensive details of a large number of applications.

There are only four chapters — Photophysical Processes in Isolated Molecules, Photophysical Processes in Molecules in Solution, Instrumentation, and Applications: references are presented at the end of each (430 for the final chapter alone). Chapter 3 contains a useful list of solvent systems (one, two, three or four components) for phosphorimetric analysis. Chapter 4 includes nearly 50 pages of tabular data for direct and indirect fluorimetric methods for organic and inorganic compounds, and for phosphorimetric methods for organic compounds; details of solvents, wavelengths, lifetimes and limits of detection are given.

Although the Pergamon system classifies this book within the research/reference category, it also contains useful introductory material for students. This book can therefore be recommended generally; the price is certainly reasonable by present standards.

D. M. W. Anderson

L. Lang (Ed.), *Absorption Spectra in the Infrared Region*, Vol. 4, Akademiai Kiado, Budapest, 1978, 318 pp., price £20 (approx.)

The comments that were offered in respect of the first three volumes in this series (Anal. Chim. Acta, 94 (1977) 222) apply equally well to this one. The style, standard and format remain unchanged: perhaps the main difference is that Butterworth's former role as joint publishers — which ended after vols. 1 and 2 — has been taken over by the Robert E. Kreiger Co., Huntingdon, New York. One of the claims made in the advertising preamble on the dust-jacket is that primary consideration has been given to the inclusion of

compounds that have been isolated or synthesized recently, but the appearance of compounds such as triethylamine, 1-heptanol, triphenylphosphine oxide, pyridinium chloride, gallic acid, and quercetin make this rather difficult to sustain. The spectra are acceptable in standard, but the range of compounds included is so diverse and random that there seems little possibility that anyone would find more than one or two spectra of specialised interest in any one volume. If the idea is to go on to turn out volumes steadily ad infinitum, this series will represent a prohibitively expensive way of acquiring purposeful access to spectra.

D. M. W. Anderson

Analytica Chimica Acta, 99 (1978) 404

Errata

D. Stojanovic, J. Bradshaw, and J. D. Winefordner, Atomic Absorption Inhibition Release Titration as a Method of Studying Releasing and Inhibiting Effects. Studies of the Mechanism of Formation of Calcium Phosphate Compounds. *Anal. Chim. Acta*, 96 (1978) 45—54.

On page 45, the last line on the page, *less stable* should be more stable.
On page 46, paragraph 3, line 6, *cation* should be anion.
On page 50, paragraph 2, lines 2 and 3, *from 0 to point c* should be from 0 to point d.

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On page 80, in eqn. (41): for $\Delta(\Delta i_{f,npp})_{max}$, read $\Delta(\Delta i_{f,npp})$.

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